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Vitrea clingmani Dall in Pilsbry, 1900, a snail endemic to the summits of the Black Mountains and Great Craggy Mountains of North Carolina is now assigned to the genus *Pilsbryna* (Gastropoda: Stylommatophora: Oxychilidae)

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ABSTRACT

Collecting in wet leaf-litter microhabitats in the Great Craggy Mountains and Black Mountains of western North Carolina uncovered juvenile shells and live adults of the rare snail *Vitrea clingmani* Dall in Pilsbry, 1900. This newly available material allows the description of the radula, juvenile shell, and reproductive system of this species, providing characters for a phylogenetic analysis, which suggests reassignment of the species from the genus *Glyphyalinia* (*Glyphyalus*) to *Pilsbryna*. This new placement is corroborated by COI sequence data. Surveys near the type locality expand the known range of *Pilsbryna clingmani* and suggest this species is endemic to spruce-fir and northern hardwood forests at elevations above 1500 m in the Black Mountains and contiguous Great Craggy Mountains of western North Carolina where it is further restricted to wet leaf litter microhabitats, making it one of the most narrowly distributed snail species in eastern North America.

Additional Keywords: Terrestrial snail, *Pilsbryna*, Appalachian Mountains, spruce-fir forest, seeps

INTRODUCTION

Pilsbryna clingmani, under the name *Glyphyalinia clingmani*, is listed as a species of concern by the U.S. Fish and Wildlife Service and a threatened species ranked as critically imperiled in North Carolina (LeGrande et al., 2008), but very little is known about its range and habitat. What little is known is based largely on reports from two brief surveys of Mount Mitchell, North Carolina, by experienced malacologists (Pilsbry, 1900; Walker and Pilsbry, 1902) which resulted in a handful of specimens now scattered among several museum collections. This scarcity of records suggests the species is not widely distributed or may require or prefer microhabitats not commonly or easily sampled. In the past century the forests of Mount Mitchell have undergone profound change and during that time *Pilsbryna clingmani* has been reported only once (Hubricht, 1970).

Non-marine mollusks have the unfortunate distinction of having the highest number of recent extinctions documented in any animal group (Régnier et al., 2009; Cowie et al., 2017). Many of these were extinctions of terrestrial snails endemic to islands but snails restricted to other geographically isolated habitats are vulnerable to habitat loss that might lead to extinction. Several of eastern North America's most narrowly distributed snail species (Hubricht, 1985) occur in southern spruce-fir forest (Pilsbry, 1940; 1946; 1948). This community is dominated by canopy species *Abies fraseri* (Pursh, 1814) and *Picea rubens* Sargent, 1898, and is limited to seven disjunct stands at peaks above 1500 m elevation in the southern Appalachian Mountains. *Pilsbryna clingmani* is known from one of these stands of southern spruce-fir forest at the summits of the Black Mountains (Walker and Pilsbry, 1902). All published records were based on specimens collected prior to 1902 within 5 km of Mount Mitchell at elevations above 1900 m. Shortly afterwards approximately 50–65% of the spruce-fir forest on the Black Mountains was logged between 1912 and 1927 (Pyle and Schafale, 1988). Loss of forest cover prompted reforestation efforts, which unfortunately included non-native trees (Minckler, 1940). These non-native species are probably the source of the catastrophic introduction of the balsam woolly adelgid, *Adelges piceae* (Ratzeburg, 1844), which was first reported on Mount Mitchell in 1957 (Speers, 1958). Nearly all mature fir trees in the Black Mountains had succumbed to *A. piceae* by 1988 (Dull et al., 1988), opening large portions of the formerly dense canopy and exposing red spruce, *Picea rubens*, to damage similar to thinning mortality (Nicholas et al., 1992). Acid mists associated with air pollution reported from Mount Mitchell (DeFelice, 1997) probably cause additional reduction in growth of red spruce by lowering availability of calcium (Jacobson et al., 1990). The more open canopy probably produces changes in soil moisture

and leaf litter cover that may negatively impact moisture-dependent land-snails.

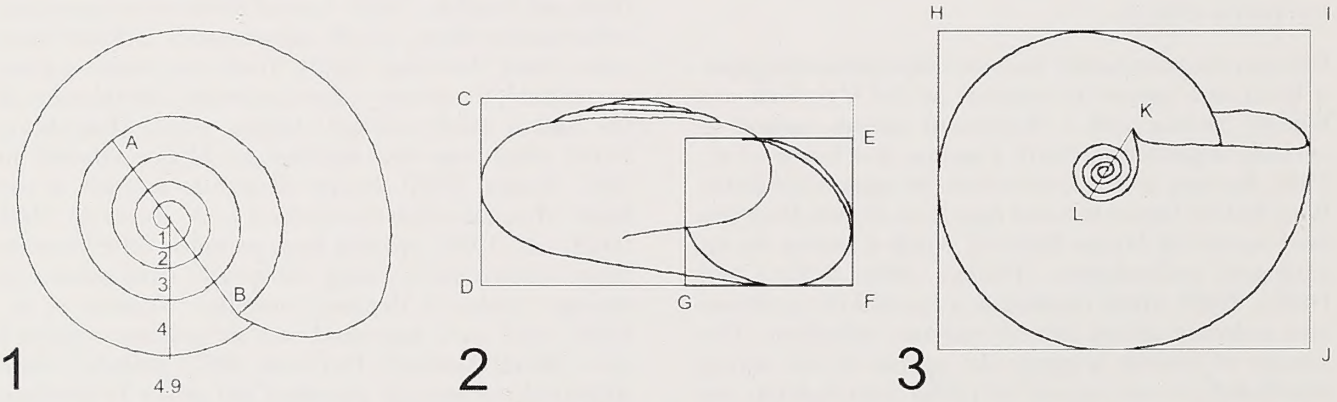
Recent land snail surveys in wet leaf litter microhabitats in the Black Mountains and contiguous Great Craggy Mountains in western North Carolina found specimens of *Pilsbryna clingmani*. Rediscovery of this species permits its redescription, comparison with other species, reassignment to the genus *Pilsbryna*, and clarification of its geographic range and habitat requirements.

MATERIALS AND METHODS

Twenty-nine survey sites that were likely to contain snails were chosen along easily accessible roadways and trails in the Black Mountains and Craggy Mountains near Mount Mitchell, with special attention given to seeps in both open and forested habitats. An additional 41 sites were sampled in other mountain ranges in western North Carolina. Specimens were hand-collected from the leaf-litter soil interface during 1 hour searches within sites of approximately 100 m^2. Additional specimens were picked from approximately 1 liter samples of leaf-litter sievings gathered from each site. Live collected animals were narcotized overnight in a suspension of water containing a 1 cm length of mentholated cigarette and then preserved in 75% ethanol. Gross anatomical dissections were made under 75% ethanol using a dissecting microscope. Isolated reproductive systems were stained with Harris' haematoxylin and Semichon's aceto-carmine stain, cleared with glycerin, and mounted on slides. Drawings of the shell and reproductive anatomy were made with the assistance of a *camera lucida*, and measurements taken using an ocular micrometer. Greater and lesser shell widths, shell height, aperture width, aperture height, umbilical width, spire width, and number of whorls were measured as described by Slapcinsky and Coles (2004) and shown in Figures 1–3. Means and standard deviations are reported for all measurements of 14 undamaged adult specimens. Shells with rapidly expanding body whorls and 4.9 or more whorls were presumed to be mature and two were dissected and confirmed to have adult reproductive structures. Radulae were isolated from dissected buccal masses using a saturated KOH solution. Scanning electron micrographs of shells and radulae were made using a HITACHI S-4000 FE-SEM. All available specimens of *Pilsbryna clingmani* in the collections of the Academy of Natural Sciences, Philadelphia (ANSP); Carnegie Museum of Natural History, Pittsburg (CMNH); Field Museum of Natural History, Chicago (FMNH); National Museum of Natural History (USNM); Schiele Museum of Natural History, Gastonia, North Carolina (SMNC); University of Florida, Florida Museum of Natural History, Gainesville (UF); and in the private collections of Harry G. Lee, Jacksonville, Florida (HGL) and Amy Van Devender (ASV) were examined. Anatomical terminology follows Tompa (1984). Higher-level systematics follows Bouchet et al. (2017).

Morphological characters derived from dissections combined with those gleaned from published figures of reproductive systems, radulae, and shells (Baker, 1929a, b; Pilsbry, 1946; Slapcinsky and Coles, 2004) were used to build a matrix of 28 characters (Table 1) for 47 oxychilid species (Table 2). Characters were treated as unordered. A phylogenetic analysis was performed using the optimality criterion of parsimony in Mesquite version 3.2 (Maddison and Maddison, 2017).

DNA was extracted from 1 mm^3 samples of foot tissue using a solution of 10% Chelex beads (Bio-Rad Laboratories) in sterile water heated to 65°C for 4 hours. DNAses and other protein contaminants were digested using Proteinase K. A 655 bp nucleotide fragment of cytochrome oxidase I (COI) was amplified using the primer pairs LCO1490/HCO2198 (Folmer et al., 1994) and GoTaq DNA Polymerase (Promega, Madison, Wisconsin). Sequencing was performed in both directions at the University of Florida, Interdisciplinary Center for Biotechnology Research or the Canadian Centre for DNA Barcoding (CCDB), using their standard procedures. Primer regions were trimmed and sequences edited and aligned using Geneious version 6.1.7 (Kearse



Figures 1–3. Shell measurements. **1.** Spire width (line a–b); whorl count (line 1–4.9). **2.** Shell height (line c–d); aperture height (line e–f); aperture width (line g–f). **3.** Greater width (line h–i); lesser width (line i–j); umbilical width (line k–l).

Table 1. Morphological characters of the shell, radula, and reproductive system used in the phylogenetic analysis.

1. shell size: 0=large, >12 mm; 1=small, <12 mm
2. whorls: 0=<3.5; 1=3.8-4.2; 2=4.5-5.5; 3=>6
3. umbilicus: 0= umbilicate; 1= perforate/imperforate
4. axial grooves: 0=absent; 1=close and irregular; 2 distant and regular
5. body whorl: 0=evenly expanding; 1=body whorl flared
6. parietal lamella: 0=absent; 1=present
7. columellar lamellae: 0=absent; 1=present
8. subbasal lamellae: 0=absent; 1=present
9. basal lamellae: 0=absent; 1=present
10. subpalatal lamellae: 0=absent; 1=present
11. palatal lamellae: 0=absent; 1=present
12. suprapalatal lamellae: 0=absent; 1=present
13. sutural lamellae: 0=absent; 1=present
14. axial fusion: 0=absent; 1=present, ?=no lamellae
15. spiral fusion: 0=absent; 1=present, ?=no lamellae
16. lamellae axial spacing: 0=not widely spaced; 1=spaced 1/4-1/2 whorl; ?=no lamellae
17. periuterine gland: 0=absent; 1=present
18. perivaginal gland: 0=absent; 1=present
19. vas deferens: 0=long; 1 short
20. epiphallar caecum: 0=absent; 1=present
21. epiphallar flagellum: 0=absent; 1=present
22. epiphallar insertion: 0=apical; 1=subapical
23. oval subapical penial pilasters: 0=absent; 1=present
24. central mesocones: 0=attenuate; 1=diamond shaped
25. lateral teeth: 0=tricuspid; 1=bicuspid
26. marginal teeth: 0=smooth; 1=serrate
27. jaw cusps: 0=three cusps; 1=four or five cusps
28. spacing of jaw cusps: 0=cusps evenly spread; 1=cusps central

et al., 2012) and deposited in GenBank under accession numbers MG648722-MG648731. Phylogenetic analysis using maximum likelihood and the GTR + I + G model of evolution was performed in MEGA6 (Tamura et al., 2013).

SYSTEMATICS

Family Oxychilidae Hesse in Geyer, 1927

Family Placement: Traditionally, most North American limacoid species have been placed in a morphologically heterogeneous Zonitidae (Baker, 1931; Pilsbry, 1946; Riedel, 1980). A cladistic analysis of Limacoidea using reproductive, muscular, nervous, lung, digestive, external body, and shell characters cast doubt on the monophyly of Zonitidae (Hausdorf, 1998) and elevated most of its component subfamilies. *Paravitrea* and its then synonym *Pilsbryna* (see Riedel, 1980), were excluded from Vitrininae, now Pristilomatidae, and moved to Daudebardiidae Kobelt, 1906. The genera *Glyphyalinia*, *Nesovitrea*, *Mesomphix*, and *Vitrinizonites* were also placed in Dauderbardiidae. More recently, Daudeberdiinae has been treated as a subfamily within Oxychilidae (Bouchet et al., 2017). However, family level relationships of taxa formerly treated as Zonitidae are not fully resolved and remain controversial (Schileyko, 2003, Welter-Schultes, 2012).

Genus *Pilsbryna* Baker, 1929

Type Species: *Pilsbryna aurea* Baker, 1929 by monotypy.

Pilsbryna clingmani (Dall in Pilsbry, 1900) new combination

(Figures 4–18)

Zonites wheatleyi.—Binney, 1885: 223. [not *Zonites wheatleyi* Bland, 1883].

V[itrea]. clingmani Dall, 1898: 100–101 [nomen nudum as a variety of *Zonites wheatleyi*].

Vitrea clingmani Dall in Pilsbry, 1900: 149–150, Figure 2; Walker and Pilsbry, 1902: 431, pl. 24, Figures 1–3.

Vitrea approxima Walker and Pilsbry, 1902: 431–432, pl. 24, Figures 7–9.

Retinella clingmani.—Pilsbry, 1946: 275–276, Figure 136a.

Retinella approxima.—Pilsbry, 1946: 276–277, Figure 137.

Glyphyalinia clingmani.—Hubricht, 1970: 13; Hubricht, 1974: 34 [synonymizes *Retinella approxima* with *Glyphyalinia clingmani*]; Hubricht, 1985: 23.

Diagnosis: A large *Pilsbryna* with an adult shell of 4.9–5.4 whorls that is 5.0–7.8 mm in width and 2.6–3.9 mm in height. Early whorls increase slowly and regularly in size but whorl expansion increases in the body whorl of the adult. Shells with fewer than 2.5 whorls contain a sinuous parietal lamella and two spirally arranged series of basal and palatal nodules that can extend a full whorl into the aperture.

Description: Body coloration dark blue-grey (Figure 4). Shell is depressed-helicoid, umbilicate, glossy, translucent, brown, and sculptured with dense and irregularly spaced, indented axial lines that become more frequent on the last quarter of the body whorl of the adult (Figures 5–8). Adult shell (n=14) 5.0–7.8 mm (mean=6.2±0.9 mm) in greater width, 4.1–6.3 mm (mean=5.0±0.7) in lesser width and 2.6–3.9 mm (mean=3.1±0.5 mm) in height with 4.9–5.4 (mean = 5.1±0.1) slowly expanding whorls. The final 1/3 of the body whorl of the adult expands more rapidly than previous whorls; the lesser/greater width ratio is 0.80–0.87 (mean=0.85). The umbilicus also expands rapidly in the body whorl of the adult, reaching a width of 0.7 to 1.0 mm (mean = 0.9±0.1). Spire width is 2.4–3.9 mm (mean=3.0± 0.4). The aperture is ovate; aperture width is 2.7–4.0 mm (mean=3.2±0.5), aperture height is 2.1–3.0 mm (mean=2.5±0.3). Shells of immature animals with fewer than 2.5 whorls have a sinuous parietal lamella and a series of paired basal and palatal nodules (Figures 9–10). All traces of nodules and lamellae are resorbed in adults.

Reproductive Anatomy: Two specimens were examined (UF 292689). The basal half of the penis is smooth while the apical half bears numerous small thorn shaped papillae (Figure 11). The penial retractor muscle is apically

Table 2. Matrix of 28 characters and 47 species used in the morphological analysis (see **Table 1** for explanation of characters).

	Characters																										
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Glyphalinia burringtoni	1	0	0	1	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	1	1	1	0	?	?
Glyphalinia carolinensis	1	2	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	1	0	1	0	1	0	?	?	?
Glyphalinia cryptomphala	1	1	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	1	0	1	0	1	0	?	?	?
Glyphalinia cumberlandiana	1	0	0	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	0	1	0	0	?	?
Glyphalinia indentata	1	0	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	?	0	1	0	1	0	1	?	?
Glyphalinia lewisiana	1	0	0	1	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	1	1	1	0	?	?
Glyphalinia paucilirata	1	0	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	1	0	1	0	1	0	1	0	1
Glyphalinia pentadelphia	1	0	0	1	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	0	1	0	0	?	?
Glyphalinia praecox	1	1	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	?	0	1	0	1	0	1	0	1
Glyphalinia rhoadsi	1	0	0	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	1	1	0	0	0	0
Glyphalinia roemeri	1	0	0	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	1	0	1	1	0	?	?
Glyphalinia sculptilis	1	2	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	1	0	1	0	1	0	1	?	?
Glyphalinia solida	1	1	1	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	1	0	1	0	1	0	?	0	1
Glyphalinia wheatleyi	1	1	0	2	0	0	0	0	0	0	0	0	0	?	?	?	1	1	1	0	0	0	0	1	0	0	?	?
Mesomphix andrewsae	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Mesomphix capnodes	0	1	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	1	0	0	0	1	0	0	0	0	?	?
Mesomphix cupreus	0	1	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	1	0	0	0	1	0	0	0	0	?	?
Mesomphix friabilis	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	1	0	0	0	1	0	0	0	0	?	?
Mesomphix inornatus	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Mesomphix latior	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Mesomphix perlaevis	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	0	0
Mesomphix pilsbryi	0	1	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	1	0	0	0	1	0	0	0	0	?	?
Mesomphix rugeli	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Mesomphix subplanus	0	2	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Mesomphix vulgatus	0	1	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	1	0	0	0	0	?	?
Nesovitrea binneyana	1	0	0	1	0	0	0	0	0	0	0	0	0	?	?	?	1	0	1	0	0	0	1	1	1	0	?	?
Nesovitrea electrina	1	0	0	1	0	0	0	0	0	0	0	0	0	?	?	?	1	0	1	0	0	0	1	1	1	0	?	?
Oxychilus cellarius	1	1	0	0	0	0	0	0	0	0	0	0	0	?	?	?	1	0	0	0	0	0	?	0	1	0	?	?
Paravitrea andrewsae	1	2	0	1	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0	1	0	0	1	1	0	1	1
Paravitrea calcicola	1	2	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	?	?
Paravitrea clappi	1	2	0	1	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea lamellidens	1	2	0	1	0	0	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	1
Paravitrea multidentata	1	2	0	1	0	0	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea petrophila	1	2	0	1	0	0	0	0	0	0	0	0	0	?	?	?	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea pilsbryana	1	2	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	?	?
Paravitrea placentula	1	2	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea pontis	1	2	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea tridens	1	2	0	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	?	?
Paravitrea variabilis	1	2	0	1	0	0	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Paravitrea walkeri	1	2	0	1	0	0	1	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	1	0	?	?
Pilsbryna aurea	1	1	0	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	?	?
Pilsbryna castanea	1	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	1
Pilsbryna clingmani	1	1	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	?	?
Pilsbryna nodopalma	1	1	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	?	?
Pilsbryna quadrilamellata	1	1	0	1	1	1	1	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	0	?	?
Pilsbryna vanattai	1	1	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	?	?
Vitrinizonites latissimus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	0	0	0	0	0	0	0	?	?

inserted. The epiphallus is about the same length and roughly 2/3 the diameter of the penis and inserts laterally at the apex of the penis. The epiphallus is sculptured with longitudinal folds and tapers slightly before widening at the junction with the narrow vas deferens. The spermatheca is ovate, and the narrow spermathecal duct expands slightly basally. No sign of perioviducal or perivaginal glands were observed. While most characters varied little in size, the albumen gland and oviduct were larger in one of the two specimens that were dissected.

Radula: Two specimens were examined (UF 292689). The central tooth of the radula is symetrically tricuspid, the mesocone expands slightly above the ectocones, then tapers slowly to the apex (Figure 12). The ectocones are short and symmetrical, less than ½ the total height of the tooth. The three laterals are tall, slender, and asymmetrically tricuspid and slightly convex proximally and concave distally. The endocone is tall, about ¾ the height of the mesocone while the ectocone is less than ½ the height of the mesocone. The marginals are tall, slender, smooth,

Figure 4. *Pilsbryna clingmani* (Dall in Pilsbry, 1900). Live individual from Mount Mitchell, Yancey County, North Carolina. Photographed by R. Wayne Van Devender.

and unicuspid, with concave peripheral edges and convex distal edges.

Holotype: USNM 38910 (Figures 13–15), from type locality.

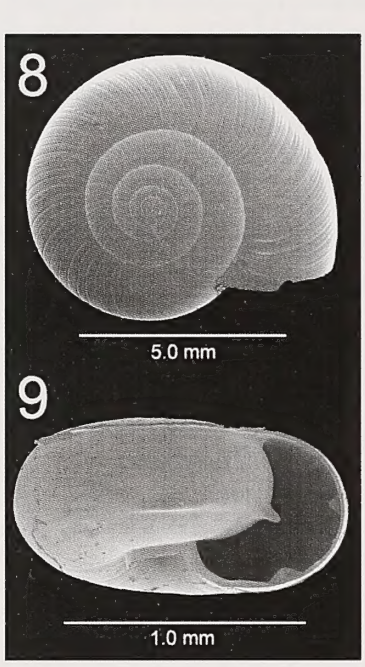
Type Locality: USA, North Carolina, Yancey County, Black Mountains near Clingman's Peak, coll. H. Hemphill, date unknown but before the publication of Binney (1885).

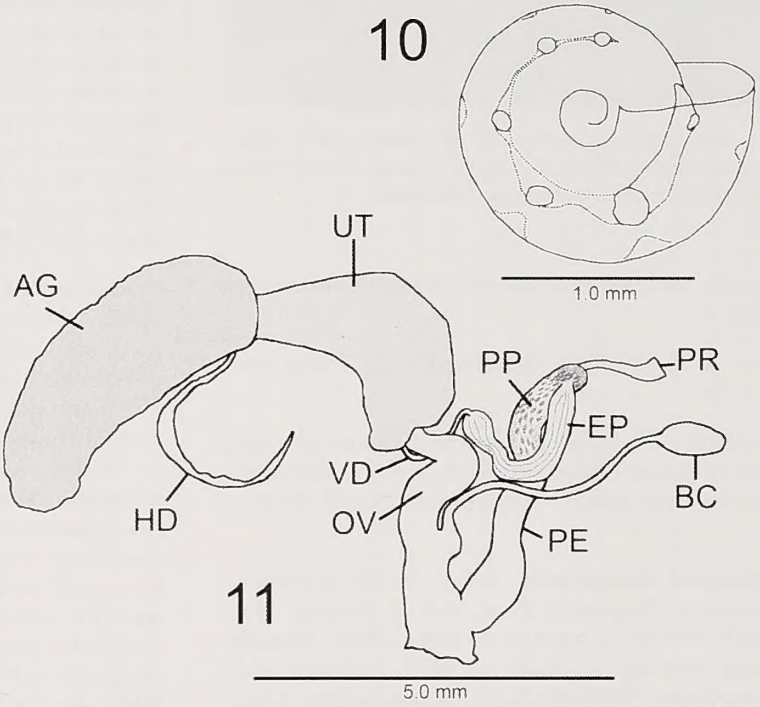
Other Material Examined: USA, North Carolina, Yancey County, Clingman's Peak, coll. J. Ferriss, May 1901: ANSP 84060, 1 specimen; CM 73964, January 1905 (month and day reversed on label, probably May 1901), 1 specimen; FMNH 58325, 4 specimens. USA, North Carolina, Yancey County, Mount Mitchell: CM 73963, coll. H. Hemphill, November 1895, 1 specimen, identified as *Retinella approxima* on the label; FMNH 240953, coll. L. Hubricht, 26 May 1962, 9 specimens; FMNH 240954, coll. L. Hubricht, 26 May 1962, 1 specimen. USA, North Carolina, Yancey County, Mount Mitchell State Park: near summit parking area, ASV-2007-184, 35.7665° N, 82.2644° W, 2001 m, coll. A. and W. Van Devender, 3 November 2007, 1 specimen; near family camping area, ASV-2008-14, 35.7599° N, 82.2711° W, 1920 m, coll. A. and W. Van Devender, 3 November 2007, 1 specimen; near campground, ASV-2011-99, 35.7600° N, 82.2709° W, 1914 m, coll. A. Gettleman and Denise Furr, 1 October 2011, 2 specimens; SMNC MAL666-01, 35.760701° N, 82.270285° W, 1915 m, coll. Denise Furr, 1 specimen. USA, North Carolina, Yancey County, Mount Mitchell, Wilson's Cove, ANSP 83260, coll. B. Walker, 1901, Lectotype (Baker, 1962) of *Vitrea approxima* Walker and Pilsbry, 1902 (Figures 16–18). USA, North Carolina, Yancey County, Potato Top: ANSP 83262, coll. J. Ferriss, 8 July 1902, 5 specimens; UF 107824, coll. J. Ferriss, 1901, 1 specimen. USA, North Carolina, Buncombe County, Bear Pen Gap near Craggy Gardens Picnic Area, 35.701° N, 82.391° W, coll. J. Slapcinsky: UF 292689, 29 May 2001, 6 specimens; UF 299560, 12 September 2002, 3 specimens; UF 341346, 19 June 2004, 1 specimen; UF 347735, 21 August 2004, 5 specimens.

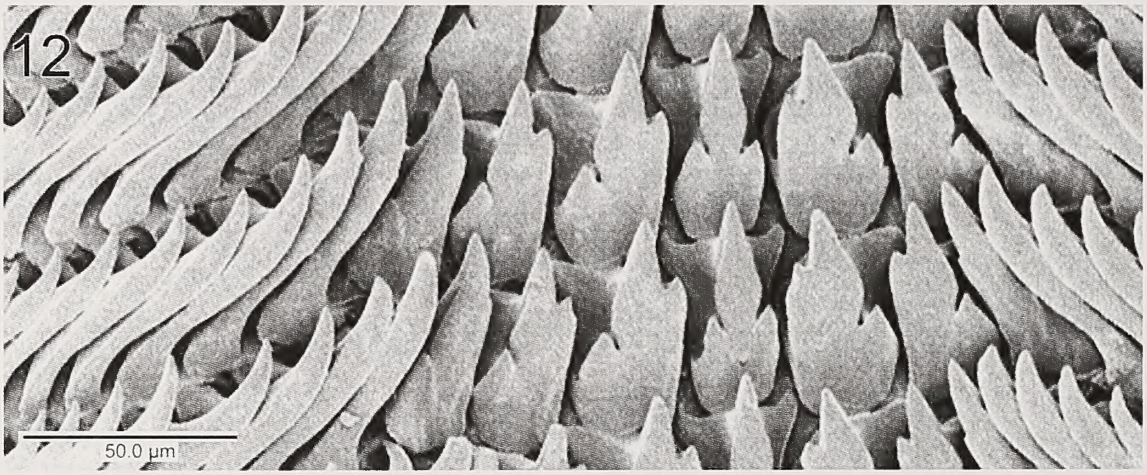
Remarks: Binney (1885) identified Hemphill's specimen from Clingham's [sic] Peak as *Zonites wheatleyi*, which is treated as a species of *Glyphyalinia* (Hubricht, 1985). Dall (1898) gave the varietal name *Vitrea clingmani* to Hemphill's shell but did not provide a figure or description. Pilsbry (1900) indicated that Dall's new name was a *nomen nudum* and included a description, a reference to a type lot, USNM 38910, and a figure, all of which were provided by Dall. In addition to the holotype, which is consistent with Dall's description, measurements, and figure (Pilsbry, 1900, Figure 2), lot USNM 38910 also contained a specimen of *Paravitrea petrophila* (Bland, 1883). This is probably the specimen Dall had compared to his new species in the original description and this shell is now USNM 38910a.

Synonymy of *Vitrea approxima* Walker and Pilsbry, 1902: *Vitrea approxima* was described based on two specimens collected by James Ferris and Bryant Walker in Wilson's Cove on Mount Mitchell (Walker and Pilsbry, 1902) and does not appear to have been collected since. One of these specimens (Figures 16–18) was deposited by Walker (ANSP 83260) and selected as the lectotype by Baker (1962) based on original measurements, but cited incorrectly as ANSP 82360. The fate of the second specimen is unknown. *Vitrea approxima* differs from *Pilsbryna clingmani*, which is larger, and *Pilsbryna vanattai*, which is smaller, by having a relatively small umbilicus and lacking a rapidly expanding body whorl. Hubricht (1974) synonymized *Vitrea approxima* with *Pilsbryna clingmani* (as *Glyphyalinia*), suggesting unique attributes of the lectotype were the result of shell damage early in life. While the lectotype (Figures 16–18) has shell damage between the second and third whorls, it does not appear to be seriously deformed. It is a juvenile of only 4.6 whorls that has not reached the stage of rapid body whorl expansion that would result in a flared body whorl and relatively large umbilicus that is common in adults of all *Pilsbryna* species (Slapcinsky and Coles, 2004) and it appears to be a juvenile specimen of *Pilsbryna clingmani*.

Habitat and Distribution: All previous records of *Pilsbryna clingmani* were from spruce-fir forest above 1800 m elevation on the peaks of the Black Mountains near Mount Mitchell, North Carolina (Walker and Pilsbry, 1902). My surveys of the Craggy Mountains (Figure 19) extend the range of *P. clingmani* south to near Craggy Gardens picnic area. This also extends the elevational range of the species down to 1500 m and into northern hardwood forest. Like other *Pilsbryna* species, *P. clingmani* is common in wet leaf-litter in seep runs and ditches on wet soil or under wood. However, at higher elevation sites on Mount Mitchell, it is also common among grasses and







Figures 5–12. *Pilsbryna clingmani* (Dall in Pilsbry, 1900). **5–7.** photographs of shell SMNC MAL666-01, width 6.2 mm, photographed by R. Wayne Van Devender. **8.** Scanning electron micrograph of apical view of adult shell UF 299560, width 6.3 mm. **9.** Scanning electron micrograph of apertural view of juvenile shell of 2.5 whorls with body whorl broken to expose internal lamellae, UF 360436, width 1.4 mm. **10.** Line drawing of juvenile shell of 2.9 whorls, UF 360436, width 1.6 mm. **11.** *Camera lucida* drawing of genitalia, UF 292689, scale bar = 5.0 mm. **12.** Scanning electron micrograph of radula, UF 292689, scale bar = 50 µm. Abbreviations: AG = albumen gland, EP = epiphallus, HD = hermaphroditic duct, OV = free oviduct, PE = penis, PG = prostate gland, PP = penial papillae, PR = penial retractor muscle, BC = bursa copulatrix, UT = uterus, VD = vas deferens.

Figures 13–18. *Pilsbryna clingmani* (Dall in Pilsbry, 1900). Shells of type material. **13–15.** USNM 38910, Holotype of *Vitrea clingmani* Dall in Pilsbry, 1900, width 6.3 mm, photographed by Paige Billin-Frye. **16–18.** ANSP 83260, Lectotype of *Vitrea approxima* Walker and Pilsbry, 1902, width 4.3 mm, photographed by Paul Callomon.

leaves in open areas (Amy Van Devender, Wayne Van Devender, and Denise Furr, personal communications).

Comparative Remarks: Placement of *Vitrea clingmani* within *Pilsbryna* is supported by a phylogenetic analysis of 28 morphological characters from 47 species of Oxychilidae (Figure 20). *Pilsbryna clingmani* has a series of parietal nodules that are fused into a parietal lamella which is resorbed in adults and has a shell of approximately 5 whorls that expands slowly at first and more rapidly in the body whorl of adults; these are synapomorphies that unite *Pilsbryna* species within Oxychilidae. Juvenile *Pilsbryna clingmani* shells bear basal and parietal nodules found in *Pilsbryna* and *Paravitrea* but not in other oxychilids.

Pilsbryna clingmani does not share certain characters found in other small shelled oxychilid genera. The lamellae of *Pilsbryna* are not grouped axially and separated by ¼ to ⅓ whorl as in *Paravitrea*. *Glyphyalinia* sensu stricto have shells textured with widely and regularly spaced axial indentations, reproductive systems with perivaginal and perioviducal glands, and radulae with serrated marginal teeth and bicuspid lateral teeth; none of these characters is found in *Pilsbryna clingmani*. *Nesovitrea* and some *Glyphyalinia* species have penises with subapical oval pilasters and shells with approximately 4 whorls that expand rapidly and consistently unlike *Pilsbryna* which have apical thorn shaped papillae and shells of 5 whorls only the last of which expands rapidly.

Both morphological (Figure 20) and molecular data (Figure 21) support placement of *Pilsbryna clingmani* within *Pilsbryna*. *Pilsbryna clingmani* has a spiral row of nodules at basal and palatal positions in addition to the sinuous parietal lamella that defines all described *Pilsbryna*. The basal nodules are not fused into a lamella as in *P. quadrilamellata*. Unlike *Pilsbryna clingmani*, palatal nodules are lacking in *P. castanea*, *P. aurea*, and *P. quadrilamellata*. Of the species with palatal nodules, *P. nodopalma* has flattened blade shaped basal and palatal nodules unlike the peg-shaped nodules in *P. clingmani*, which are similar to those of the much smaller *P. vannattai*.

DISCUSSION

My survey is the first to report *Pilsbryna clingmani* from northern-hardwood forest in the Craggy Mountains, indicating that the species is not restricted to the Black Mountains or to spruce-fir forest. However, this species was only observed at elevations above 1500 m and was not found in well-sampled cove-forest at lower elevations in the same mountain ranges, where it is replaced by *P. vanattai* (Slapcinsky and Coles, 2001). Furthermore, the species was not found in 41 sites sampled outside of the Black Mountains and Craggy Mountains and there are no other records of the species outside of these mountain ranges (Hubricht, 1985). The majority of the distribution of *P. clingmani* occurs in spruce-fir forest which has experienced high mortality as a result of 60 years of adelgid impact (Dull et al., 1988). However, *P. clingmani* is still commonly encountered in damaged spruce-fir forest near the summits of the Black Mountains and it appears that the paucity of observations of this species in the past century are the result of low sampling effort. Surprisingly, *P. clingmani* is common among grass and leaf litter in open areas near the summit of Mount Mitchell (see above). These exposed habitats are normally too dry for *Pilsbryna* species (Slapcinsky and Coles, 2004). It is possible that high elevation mist on Mount Mitchell maintains soil moisture that is necessary for this *Pilsbryna* species even in open habitats. Additional sampling is necessary to determine if *P. clingmani* also inhabits other high elevation open habitats such as heath balds. The occurrence of this species in acidic leaf-litter with high

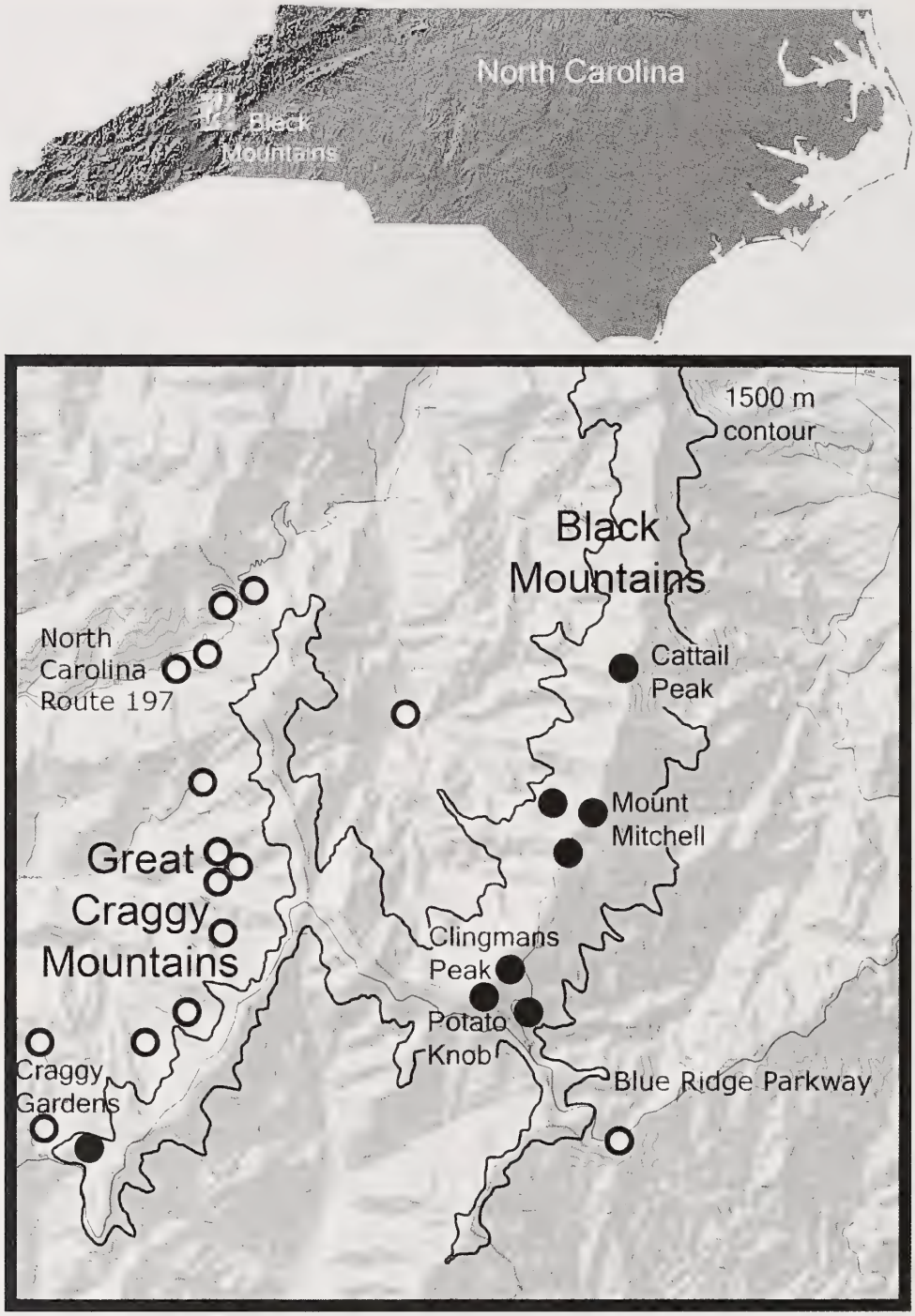


Figure 19. *Pilsbryna clingmani* (Dall in Pilsbry, 1900). Species distribution in the Great Craggy and Black Mountains, western North Carolina. Positive sites denoted by solid dots and negative sites by unfilled dots. 1500 m contour indicated.

rates of soil leaching on acid igneous rocks in conifer forest and open habitats suggests it is an acidophile (Nekola, 2010). Ecological data are needed to determine the moisture, cover, and microhabitat requirements of this rare species. This is especially important because global warming is likely to reduce isolated high elevation spruce-fir and northern-hardwood forest habitats in the southern Appalachian Mountains. The narrow distribution of *P. clingmani* in wet microhabitats above 1500 m on the peaks of the Great Craggy and Black Mountains make this species especially vulnerable and a species of special concern to conservation biologists.

Oxychilidae in eastern North America comprises six genera: the large shelled *Mesomphix* and *Vitrinizonites*

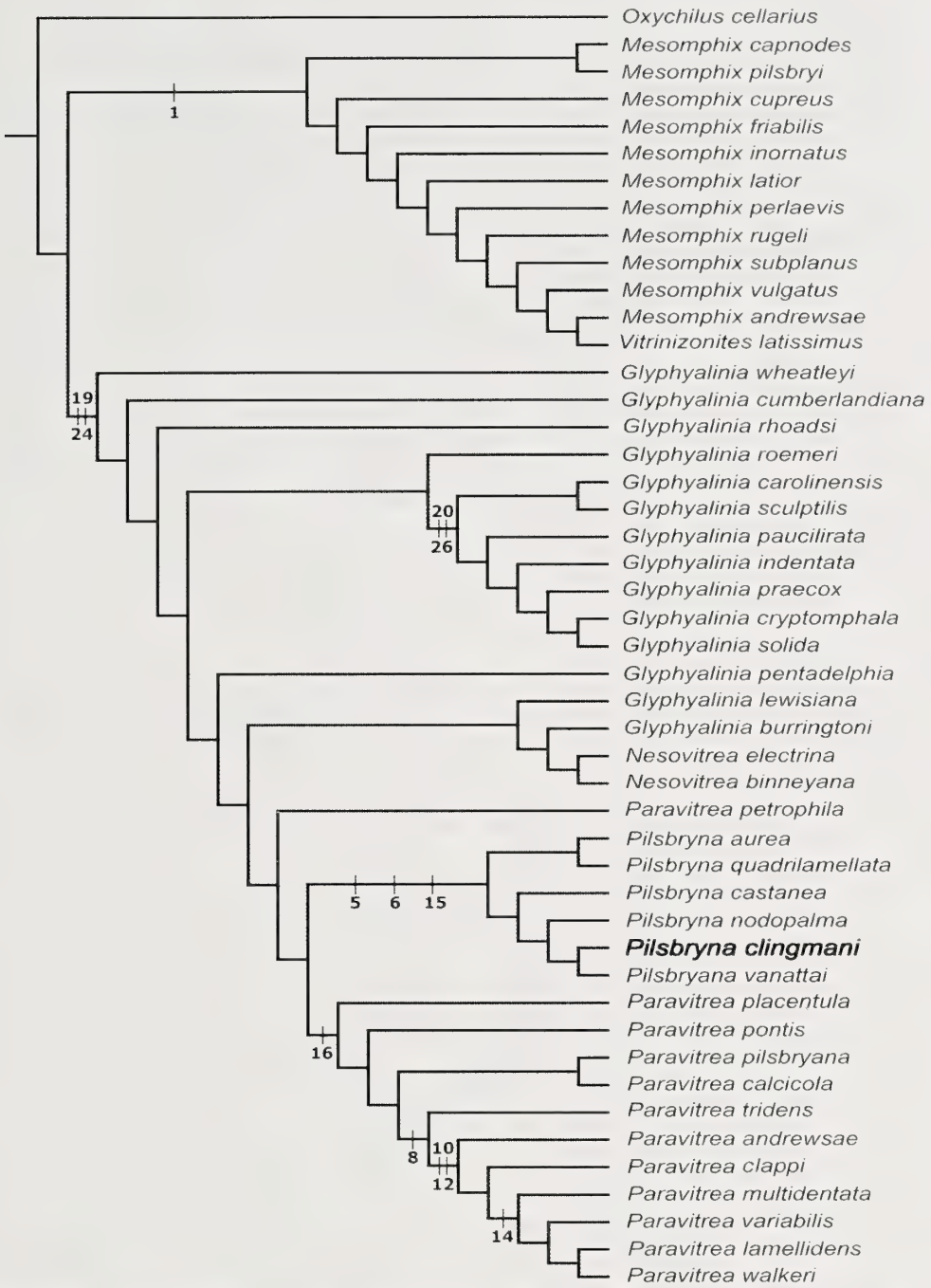


Figure 20. Majority rule consensus tree of 100 most parsimonious trees based on 28 characters from shell, radula and reproductive system. Synapomorphies are marked in bold on branches.

that are usually > 20 mm in shell width and the much smaller *Glyphyalinia*, *Nesovitrea*, *Paravitrea*, and *Pilsbryna*, which are usually < 10 mm in shell width. Unlike most of the other genera, *Pilsbryna* is morphologically well defined, composed of morphologically similar species with similar habitats in reliably moist leaf litter microhabitats. The other small bodied genera *Glyphyalinia*, *Nesovitrea* and *Paravitrea* are morphologically and ecologically diverse and probably require taxonomic revision using additional morphological and molecular characters.

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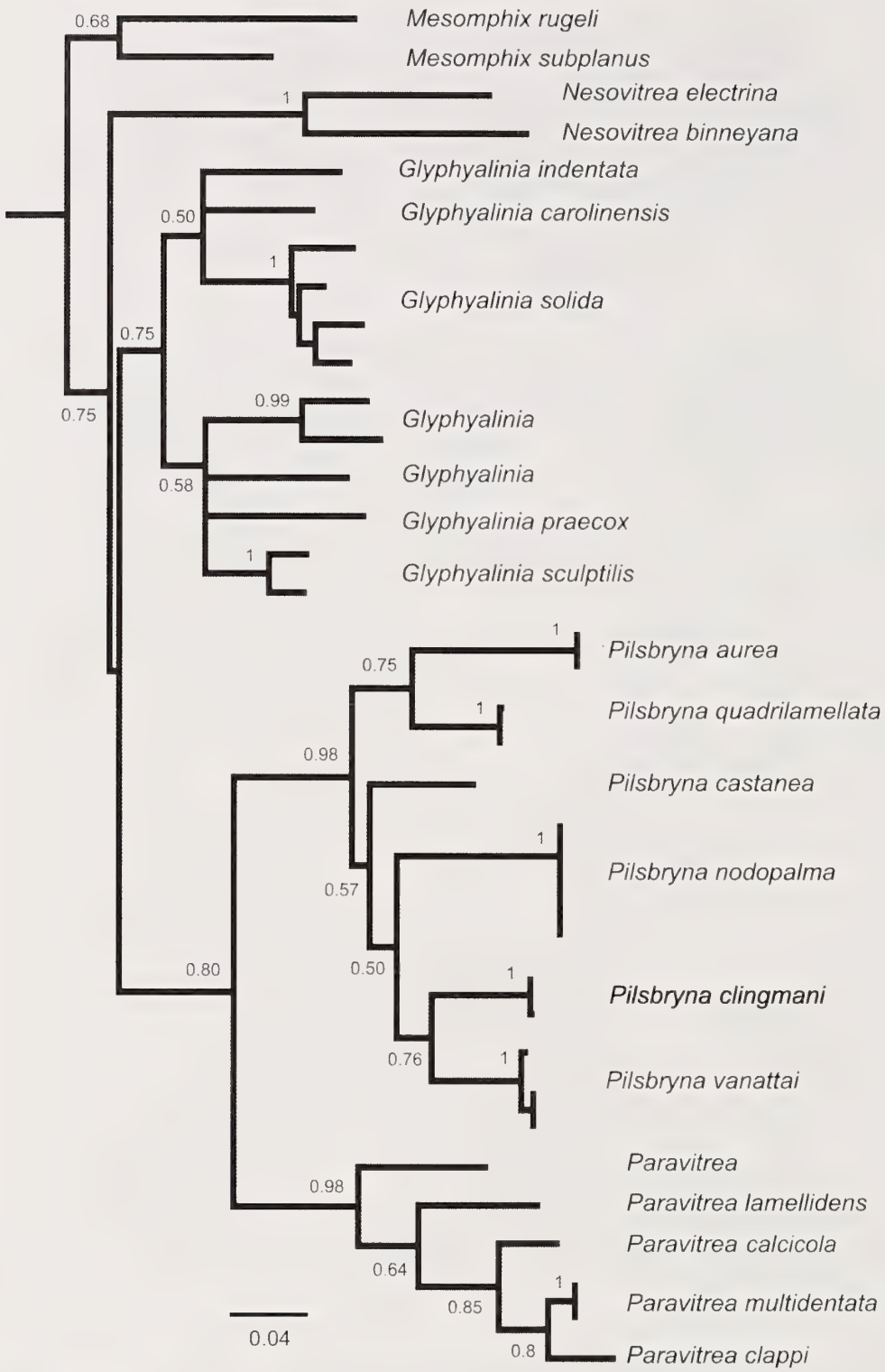

Figure 21. Phylogenetic tree based on maximum likelihood analysis of COI sequence data with node support based on 100 bootstrap replicates.

(CMNH) lent specimens or facilitated visits to collections in their care. Harry G. Lee and Wayne and Amy Van Devender loaned material from their private collections. Amy and Wayne Van Devender, and Denise Furr kindly shared unpublished habitat data and photographs from their surveys of Mount Mitchell State Park and provided comments on earlier drafts of this paper. Further improvements were suggested by reviewers Robert Cowie and Jeffrey Nekola.

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Notice

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The Bailey-Matthews National Shell Museum is pleased to invite applications for the 2018 R. Tucker Abbott Visiting Curatorship. The Curatorship, established originally in accordance with the wishes of the late Dr. R. Tucker Abbott, Founding Director of the Shell Museum, is awarded annually to enable malacologists to visit the museum for a period of one week. Abbott Fellows are expected, by performing collection-based research, to assist with the curation of portions of the Museum's collection and to provide one talk for the general public. The Museum collection consists of marine, freshwater, and terrestrial specimens. The entire collection has been digitally catalogued and the catalogue is available at the Museum web site at https://www.shellmuseum.org/malacological-collection and via iDigBio at http://ipt.idigbio.org/resource?r=bmnsm-shell. The R. Tucker Abbott Visiting Curatorship is accompanied by a stipend of $1,500.

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Sanibel, FL 33957 USA
jleal@shellmuseum.org

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Two deep-sea *Calliotropis* species (Gastropoda: Calliotropidae) from the western Pacific, with the description of a new species

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ABSTRACT

Examinations of materials deposited in the Marine Biological Museum, Chinese Academy of Sciences (MBMCAS) revealed two deep-sea calliotropid species. *Calliotropis infundibulum* (Watson, 1879), trawled from an abyssal plain in the South China Sea (17°15′ N, 111°20′ E) at a depth of 1238 m, is a new record of the species for Chinese waters. *Calliotropis yapensis* new species, collected at the Yap Seamount (8°55′ N, 137°48′ E), at a depth of 1119 m, is distinguished from its congeners by having a globular shell (height/width ratio=0.95), sculptured with granular spiral cords and prominent, densely spaced axial ribs. Phylogenetic analysis using 633 bp of the cytochrome oxidase *c* subunit I (COI) gene of *Calliotropis yapensis* new species supports its systematic placement within the genus.

Additional Keywords: Mollusca, Vetigastropoda, Seguenzioidea, South China Sea, Yap Seamount

INTRODUCTION

Based on the distinctive shell morphology and radular features, Hickman and McLean (1990) established Calliotropidae (as the tribe Calliotropini) to group some species previously under the Margaritinae, Angariinae, Monodontinae, Calliostonmatinae and Solariellinae. Members of the family are very characteristic among the Seguenzioidea by having a thin shell with an unthickened outer lip, aperture without denticles or lirae, radula with a reduced rachidian and three lateral teeth per half row. *Calliotropis* Seguenza, 1903 is the most speciose genus in this family, with more than 150 valid Recent species predominantly distributed in the Indo-West Pacific region (e.g. Australia: Jansen, 1994; New Zealand: Marshall, 1979; Solomon Islands: Vilvens, 2007; Japan: Okutani, 2000; Philippines: Poppe et al., 2006; China: Vilvens, 2007; Indonesia: Hickman, 2016).

Although many surveys have been carried out during the last few decades (e.g. Bartsch, 1942; Bouchet and Warén, 1985; Bouchet and Poppe, 1988; Marshall, 1988; Okutani and Iwasaki, 2003; Kantor et al., 2013), the knowledge of deep-water malacofauna of the western Pacific is still far from sufficient. Almost every survey is bound to discover new taxa and great numbers of new distributional records. Over the past decade, more than 50 *Calliotropis* species have been described as new from the deep waters of the western Pacific region (Poppe et al., 2006; Vilvens, 2006; 2007; Hickman, 2016). These findings strongly suggest that many more new deep-sea species are awaiting description.

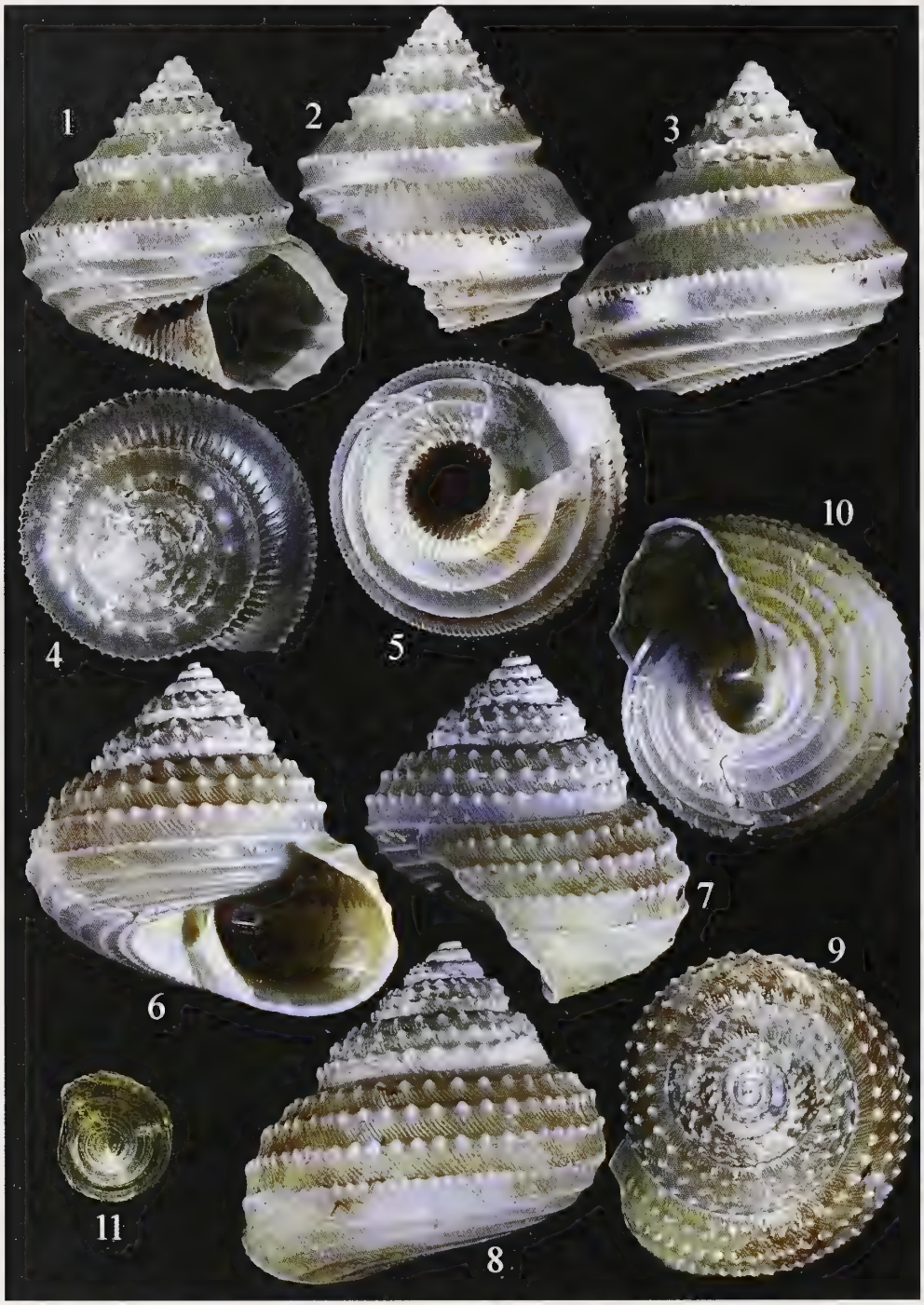
In recent years, several deep-sea surveys have been carried out by Institute of Oceanology, Chinese Academy of Sciences (IOCAS). During these surveys, two species of *Calliotropis* were sampled: one represents a new record for Chinese water and another one belongs to an undescribed species. In present study, we describe and illustrate these two species.

MATERIALS AND METHODS

The studied specimens of *Calliotropis infundibulum* (Watson, 1879) were trawled from the South China Sea, whereas the specimen of *Calliotropis yapensis* new species was collected by the mechanical arm of the ROV Faxian (based on mother-ship R/V Kexueyi) from Yap Seamount. Specimens are deposited in the Marine Biological Museum, Chinese Academy of Sciences (MBMCAS).

Morphological Methods: Shell morphology was observed under a stereomicroscope, and photos taken using a digital camera. The soft parts of the new species were retracted too deep inside the shell. Thus, it would be difficult to extract the soft parts and consequently the radula, without breaking the shell. Shell measurements were taken using a caliper with accuracy of 0.1 mm.

Molecular Procedures: One specimen of *Calliotropis yapensis* new species was used for molecular analysis.



Figures 1–11. *Calliotropis* species. **1–5** *Calliotropis infundibulum* (Watson, 1879), MBM280611, 9.4×9.2 mm, South China Sea. **6–11.** *Calliotropis yapensis* new species, Holotype MBM285093, 25.0×26.2 mm, 8°55′ N, 137°48′ E, Yap Seamount. **6–10.** Different views of shell. **11.** Operculum, maximum diameter 10.5 mm.

Genomic DNA was extracted with the Column Genomic DNA Isolation Kit (Beijing TIANGEN, China) according to the manufacturer instructions. DNA was eluted in elution buffer and stored at -20°C until use. The COI region was amplified by polymerase chain reaction (PCR) using the primers LCO1490 (forward: 5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (reverse: 5′-TTAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994). PCR reactions were carried out in a total volume of 50 μL, including 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1 μM of both forward and reverse PCR primers, 10×buffer and 2.5 U Taq DNA polymerase. Thermal cycling was performed with following conditions: 95°C for 3 min (initial denaturation), followed

by 35 cycles of 95°C for 30s (denaturation), 40°C for 30s (annealing), 72°C for 60s (extension) and a final extension at 72°C for 10min. PCR products were verified on a GelRed-stained 1.5% agarose gel and purified with the Column PCR Product Purification Kit (Shanghai Sangon, China). Purified products were sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (ver. 3.1, Applied Biosystems) and an AB PRISM 3730 (Applied Biosystems) automatic sequencer. The sequence has been deposited in GenBank (Accession number: MF499154). Sequence alignments were generated using Clustal X (Larkin et al. 2007). Neighbor-joining (NJ) tree was performed by MEGA 6.06 (Tamura et al. 2013), using Kimura 2-parameter (K2P) model (Kimura, 1980). Bootstrap analyses were performed with 1000 replications.

SYSTEMATICS

Superfamily Seguenzioidea Verrill, 1884

Family Calliotropidae Hickman and McLean, 1990

Genus *Calliotropis* Seguenza, 1903

Type Species: *Trochus ottoi* Philippi, 1844. Pliocene-Pleistocene, Italy, by original designation.

Calliotropis infundibulum (Watson, 1879) (Figures 1-5)

Trochus infundibulum Watson, 1879: 707–708.

Trochus (*Margarita*) *infundibulum*: Watson, 1886: 84, pl. 5, fig. 5.

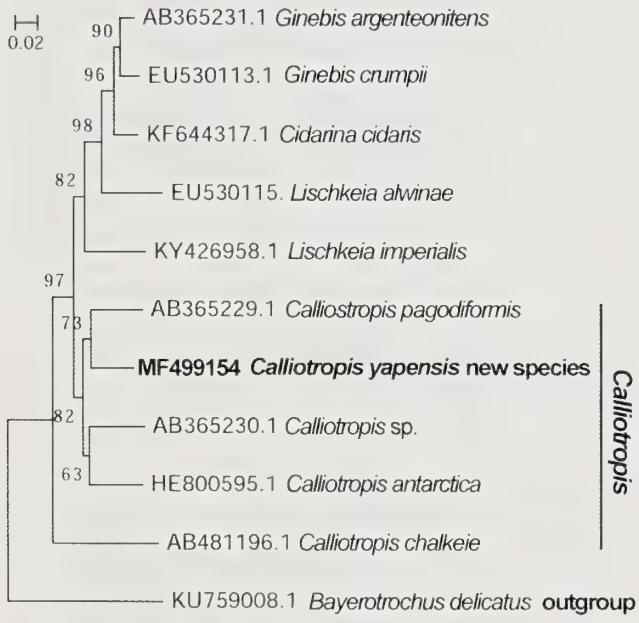


Figure 12. Neighbour-joining tree for Calliotropidae based on available COI sequences from this study and GenBank. Numbers above branches indicate the bootstrap values.

Solariella infundibulum: Dall, 1889a: 380–381; Abbott, 1974: 41, fig. 287; Cernohorsky, 1977: 105, fig. 1.

Calliotropis infundibulum: Marshall, 1979: 531, figs. 4E–G, 9C–F; Okutani, 2000: 59, pl. 29, fig. 25.; Vilvens, 2004: figs. 27–28; Vilvens, 2007, figs. 84–85.

Description: Shell (Figures 1–5) conical (H/W=1.0), very thin (aperture margin 0.2 mm thick), with 7 whorls, low-spired, spire 36% of shell height. Shell surface nacreous, indicative of exfoliation of outer shell layer that probably occurs during collection and/or preservation. Suture strongly impressed. Spire whorls angularly convex, with two beaded spiral cords separated by wide, smooth interstice. An additional spiral cord occurring above periphery of body whorl. Axials obsolete but still visible near spiral cords. Basal area with 4 spiral cords, separated by regularly spaced interstices. Interstice region sculptured with numerous oblique, curved axial riblets. Umbilicus deep, wall sculptured with prominent axial ribs.

Material Examined: Two empty shells, MBM280611, South China Sea, 17°15′ N, 111°20′ E, 1238 m deep, sandy bottom, collected by Agassiz trawl from the abyssal plain (Mother-ship R/V Kexueyi, IOCAS), 13 September 2011.

Distribution: Atlantic North America (Abbott, 1974); Caribbean (Watson, 1886); Brazil (Dall, 1889); South Africa (Martens, 1903); Marion Island (Prince Edward Islands), Atlantic-Indian-Antarctic Basin (Watson, 1879); north-eastern New Zealand (Marshall, 1879), Japan (Okutani, 2000) and China (this study). Depth range 230–3259 m (Clarke, 1962).

Remarks: No significant morphological differences could be found to separate the studied specimens from the photographs of the holotype (Cernohorsky , 1977) and syntypes (Vilvens and Swinnen, 2008).

Calliotropis infundibulum (Watson, 1879) (Type locality: Prince Edward Island, Indian-Atlantic Ridge area, 46°46′ S, 45°31′ E, 2514 m) is regarded as broadly distributed throughout the Indo-Pacific and tropical western Atlantic. However, the eastern Pacific barrier and the Mid-Atlantic barrier, and the presence of cold waters around polar or temperate margins of continents, have played important vicariant roles in the evolutionary history of tropical marine taxa (Duda and Kohn, 2004). These vicariant factors suggest that the specimens identified as *Calliotropis infundibulum* from Indo-West Pacific are probably not conspecific with those from the Atlantic. Previous authors compared the specimens from the two localities, and found no reason for further separation (Marshall, 1979; Vilvens and Swinnen, 2008). These studies, however, have mainly focused on shell morphology and lack detailed information on anatomical and molecular characters, although a radula from New Zealand was described by Marshall (1979) and a soft part from America described by Dall (1889b). Consequently, identification of Indo-Pacific specimens must be tentative until

supporting antomical and molecular data are available for comparison.

At a morphological level, although some variations exist among the shells from different localities, e.g., shell size varies from 15.3 mm to 23.5 mm, spire depressed to high, suture shallowly to deeply compressed, etc., serial intermediate forms may present (Vilvens and Swinnen, 2008). The main sculpture characters of this species are relatively consistent, e.g., spire whorl with 2 granular spiral cords and body whorl with 3 ones, with the adapical one strongest, axial ribs obsolete but still visible near the granules; basal area with 4 thin granular spiral cords, the innermost one with strong nodules; umbilicus broad, deep, with axial ribs, no spiral cords.

To date, about five species of the genus have been reported from the South China Sea including *Calliotropis annonaformis* Lee and Wu, 2001, *Calliotropis scalaris* Lee and Wu, 2001 and *Calliotropis chunfuleei* Chino, 2014 from Pratas Island at depths of 400–500 m, *Calliotropis philippei* Poppe, Tagaro, and Dekker, 2006 from off the Philippines at depths of 550–884 m, and *Calliotropis helix* Vilvens, 2007 from Taiwan at depths of 790–904 m. Among them, *Calliotropis helix* most resembles the present studied specimens in general shape, but differs in having distinct sculpture and a much more depressed body whorl. *Calliotropis infundibulum* (Watson, 1879) is recorded for the first time from the coast of China.

Calliotropis yapensis new species
(Figures 6–11)

Description: Shell (Figures 6–10) solid (aperture margin ~1.0 mm thick), large for the genus, 25.0 mm high and 26.2 mm wide (height/width=0.95), umbilicate; spire low, broadly conical, height 66% of width; suture moderately impressed. Body whorl globular, occupying 2/3 of shell length. Exterior shell layer very thin and translucent, underlying nacre visible. A very thin, brownish intritacalx well preserved on body whorl. Protoconch unknown. Teleoconch of 6 regularly expanding convex whorls. Subsutural ramp broad and ornamented by axial ribs. Sculpture of granular spiral cords with prominent, densely packed prosocline axial ribs between interstices. Intervals between ribs twice as broad as ribs. In addition to three spiral cords on spire whorl, a fourth finely granular spiral present, concealed by suture throughout growth, becoming exposed on periphery of body whorl. Nodules very pronounced, rounded or slightly sharp. Number of nodules on penultimate whorl: 24 on first cord, 25 on second cord, 30 on third cord; number of nodules on body whorl: 33 on first cord, 38 on second cord, 45 on third cord, 75 on fourth cord. Basal area with 6 finely beaded spiral cords, innermost one strongest, bordering the umbilicus, interstices between cords with secondary axial riblets. Umbilicus wide, fully covered by reflected projection of parietal and columellar callus, wall sculptured with thin axial riblets. Aperture subquadrate, 1.2 times higher than spire, backward-sloping, inside yellowish with iridescence. Parietal lip with a very thin, translucent callus. Operculum (Figure 11) paucispiral, sub-circular in shape, with central nucleus.

Type Locality: Yap Seamount, 8°55′ N, 137°48′ E, foraminiferal ooze, 1119 m depth.

Type Material: Holotype. MBM285092, collection number: Y30057, station: FX-DIVE 18, Yap Seamount, 8°55′ N, 137°48′ E, foraminiferal ooze, 1119 m deep, 18 December 2014.

Distribution and Habitat: Only known from the type locality, living on foraminiferal ooze bottom.

Etymology: The name of new species refers to its type locality.

Remarks: *Calliotropis yapensis* new species is characterized by a large shell with convex teleoconch whorls, shell surface sculptured with granular spiral cords and fine, dense, regularly spaced axial ribs. These features can easily separate new species from most species of the genus. *Calliotropis yapensis* new species is in general shell shape most similar to *Calliotropis ammos* Vilvens, 2012 from French Polynesia, which also has low spire and rounded body whorl. *Calliotropis ammos* Vilvens, 2012, however, differs from the new species in having a smaller shell (height 22.4–24.5 mm, width 21.4–23.8 mm) with a larger number of teleoconch whorls (6.9–7.1), and a lower aperture (height/height of the aperture 2.55–3.03 vs. 2.27 in new species). In addition, *Calliotropis yapensis* new species can be separated from *Calliotropis ammos* Vilvens, 2012 in having a very thin exterior shell layer through which the underlying nacre can be seen, 6 instead of 5 spirals on the base, prominent axial ribs present between the spirals, and an aperture that not transversely elongated (Vilvens, pers. comm.). *Calliotropis blacki* Marshall, 1979 and *Calliotropis derbiosa* Vilvens, 2004 may be also confused with *Calliotropis yapensis* new species, but differ in

Table 1. Pairwise distances among species of *Calliotropis* based on Kimura 2-parameter model.

		1	2	3	4
1	MF499154 *Calliotropis yapensis* new species				
2	AB365229.1 *Calliotropis pagodiformis*	0.092			
3	AB365230.1 *Calliotropis* sp.	0.117	0.117		
4	AB481196.1 *Calliotropis chalkeie*	0.192	0.197	0.199	
5	HE800595.1 *Calliotropis antarctica*	0.100	0.120	0.105	0.197

having much more angulate teleoconch whorl and depressed body whorl.

Molecular Analyses: One sequence for the COI region of *Calliotropis yapensis* new species was obtained. After manual adjustment, the length of the partial COI sequence of new species is 633 bp. The Neighbor-joining (NJ) tree (Figure 12) was reconstructed using available COI sequences from this study and GenBank. The alignment of COI had a total 594 bp, without insertions and deletions. The NJ tree shows that the new species fall into the genus *Calliotropis* in which *Calliotropis yapensis* new species together with other *Calliotropis* spp. form a well-supported clade, supporting its placement within *Calliotropis*. The phylogenetic tree also supports monophyly of a group of 5 *Calliotropis* species within the family. Within the available sequences from GenBank, *Calliotropis yapensis* new species is closest related to *Calliotropis pagodiformis*, with 9% pairwise distance, whereas the pairwise distances among *Calliotropis yapensis* new species and other species of *Calliotropis* are 10–12% (see Table 1).

In addition to the present new species, other two basal (Vetigastropoda) species were collected together during the survey, including a pleurotomariid species, *Bayerotrochus delicatus* Zhang, Zhang and Wei, 2006, and a undescribed colloniid species, *Homalopoma* sp. The three species had never yet been found elsewhere, and thus were considered potential Yap Seamount endemics.

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Redescription of the jumping snail *Ovachlamys fulgens* (Gude, 1900) (Gastropoda: Helicarionoidea: Helicarionidae): An anatomical and conchological approach

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ABSTRACT

The geographical distribution of the jumping snail *Ovachlamys fulgens* Gude, 1900, originally described from Loo-Choo Island, is expanding rapidly throughout the tropics. The full extent of the environmental damage caused by the introduction of this exotic species is still unknown. So far, it has been implicated in damage to orchids and horticultural plants. *Ovachlamys fulgens* is only known by a few characteristics of the shell and information on its anatomy is scant. The present study aims to redescribe this species based on specimens from Brazil, which is here characterized by the a globose shell, with wide aperture, externally micro-sculptured with undulating spiral groves, umbilicus partially obliterated by a thin plate, pedal sole tripartite, cephalopedal mass consisting of a series of oblique muscles, mantle with two flattened lobes, large caudal horn, pallial cavity small, vascular system evident, auricle fibrous, ventricle highly muscular, aorta subdivided in cephalic and gastric veins, a large kidney internally folded, jaw smooth and crescentic, radulae with 127 teeth per row (55-(8)-1-(8)-55), each row with about 25 μm, crop absent, salivary glands fused, stomach large with thin walls, ovotestis with at least three distinct lobes, talon totally immersed in the albumen gland, uterus with two regions, capsule gland hardly visible, *bursa copulatrix* small and sacculiform, penial sheath present, epiphallus small and narrow, nervous ring asymmetrical, visceral ganglion on left side only, five and six nerves running from each cerebral and pedal ganglia, respectively, one statocyst immersed in each pedal ganglion, and two pairs of ganglia (mandibular and buccopharyngeal ganglia) extra to the nervous ring.

Additional Keywords: Atlantic Forest, exotic species, shell morphometrics, PARNASO

INTRODUCTION

The Helicarionoidea Bourguignat, 1877 is a diverse group of pulmonate gastropods usually found in Africa, Asia, and Australasia (Smith et al. 2002). Anatomical data for many lineages in the Helicarionoidea is unknown, the phylogenetic relationships of those lineages are poorly understood, and the taxonomic concept for many families is unclear (Hyman and Ponder, 2010). The key characters considered to discriminate families are anatomical (e.g., stimulator, epiphallic caecum, flagellum) which seem to have been reduced or lost several times in many groups (Hausdorf, 1998; Schileyko, 2002a, b; Hyman, 2007; Hyman and Ponder, 2010).

Among the Helicarionoidea, *Ovachlamys fulgens* (Gude, 1900) is known from shell characters and poor anatomical data from reproductive system (Schileyko, 2002a, b). This species was originally described from Loo-Choo Island, Japan. So far, this is considered the place of origin for this species, but its distribution is expanding rapidly throughout the tropics, most likely unintentionally, as a result of the horticultural trade (Robinson and Slapcinsky, 2005). *Ovachlamys fulgens* has already been recorded in some Pacific islands (O'ahu and Hawai'i islands, in the Hawaiian Archipelago and Tutuila and Olosega, in American Samoa), in Brazil (São Paulo State), in Trinidad and Tobago, Costa Rica, and Florida (Barrientos, 1998, 2000; Cowie, 2001; Robinson and Slapcinsky, 2005; Cowie et al., 2009; Teixeira et al., 2017). It has also been reported in horticultural shipments from Thailand, Singapore, and Colombia, but identification still lacks proper confirmation (Robinson, 2003; Robinson and Slapcinsky, 2005).

Ovachlamys fulgens is considered an important pest to orchids and it is able to attack horticultural plants (Stange, 2004). The environmental damage caused by the introduction of this exotic species is still unknown. The main objective of this paper is to redescribe and illustrate shells of type material and anatomy of recently collected material of *O. fulgens* in a Brazilian national park, in Rio de Janeiro State. With this redescription, we hope to provide a baseline for future comparative

Figure 1. *Ovachlamys fulgens*. Locality of PARNASO (red star), where the field work was conducted in Rio de Janeiro State, Brazil.

studies among Helicarionoidea and help understand the biology of this exotic species, which has potential to become distributed worldwide in the tropics and subtropics and cause serious damage to environment and horticultural activities.

MATERIALS AND METHODS

The specimens from Brazil were collected in Parque Nacional da Serra dos Órgãos (PARNASO), a national park with 20,024 ha of Atlantic Forest biome. This conservation area is located in Rio de Janeiro State (Figure 1) and encompasses part of the municipalities of Guapimirim, Teresópolis, Petrópolis, and Magé. PARNASO presents an altitudinal gradient ranging from 200 to 2,263 m above sea level, with average annual temperature of 13–23° C, and average annual precipitation reaching about 2,800 mm/year, with the highest values between November and March (~ 360 mm/month) (ICMBIO, 2008).

Specimens were hand-collected, mainly around the park headquarters in Teresópolis and Guapimirim at low elevations (400–950 m a.s.l.) from October 2015 to August 2017, totaling 13 field trips (2–3 days each). Specimens were drowned in distilled water for 24h and then preserved in 70% ethanol. Voucher specimens are deposited at the Biological Institute of Universidade Federal do Rio de Janeiro (IBUFRJ) according to authorization provided by Chico Mendes Institute for Biodiversity Conservation (ICMBIO).

Taxonomic identification was based on conchological comparisons with type material and the original description and illustration. Twenty-two undamaged shells (2 shells of syntypes and 20 additional recently collected shells deposited at IBUFRJ 21236) were selected for morphometric analysis, based on size, grading from the smallest juvenile to the largest adult shell. Six measurements were taken as descriptors of morphological variation (Figure 2): shell height (H), shell width (W), height of spire (Hs), height of last whorl (Hl), height of aperture (Ha), width of aperture (Wa). Also,

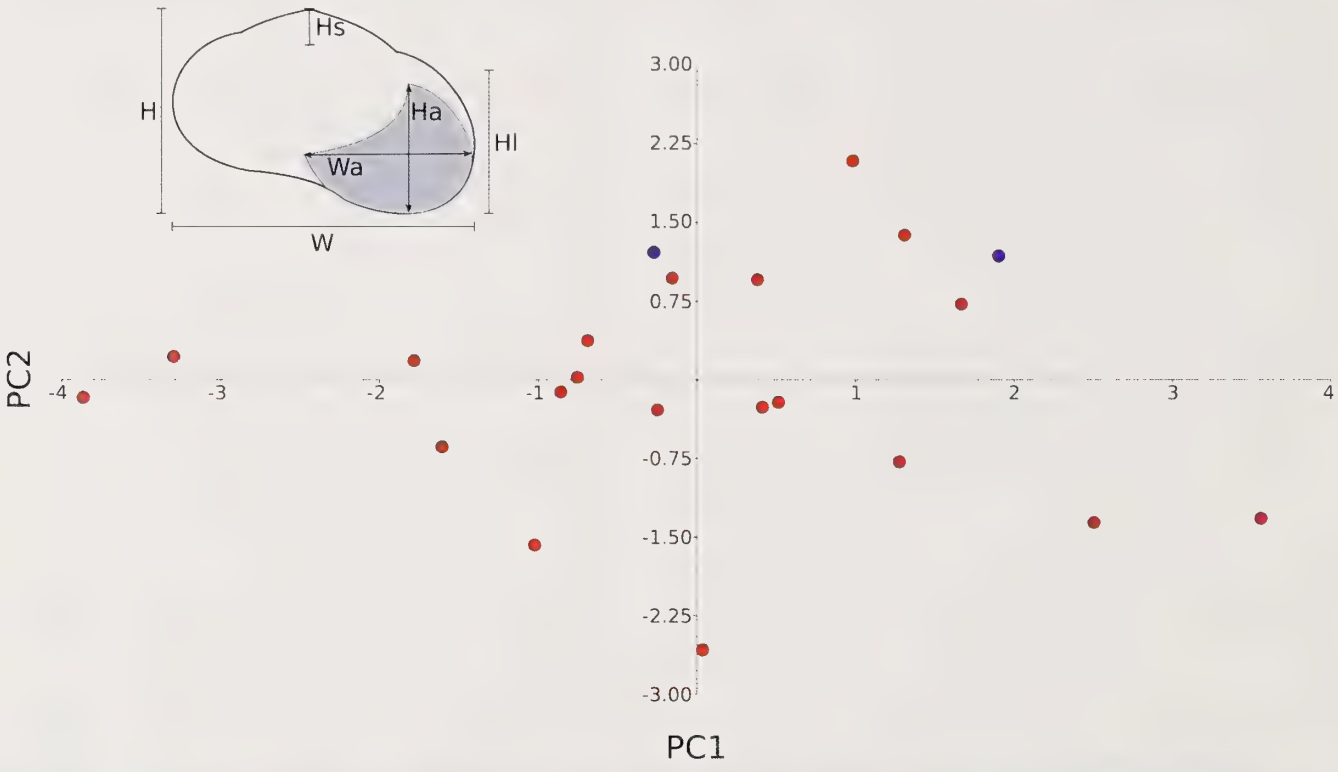


Figure 2. *Ovachlamys fulgens*. Shell descriptors and the scatter plot of 22 studied specimens along PC1 and PC2 based on 11 quantitative features. Syntypes in blue.

Figures 3–6. *Ovachlamys fulgens*. Shells in apical and apertural view. **3–4.** Syntype (NHMUK 1922.8.29.12). **5–6.** Specimens from Brazil (IBUFRJ 21236). Scale bar =1 mm.

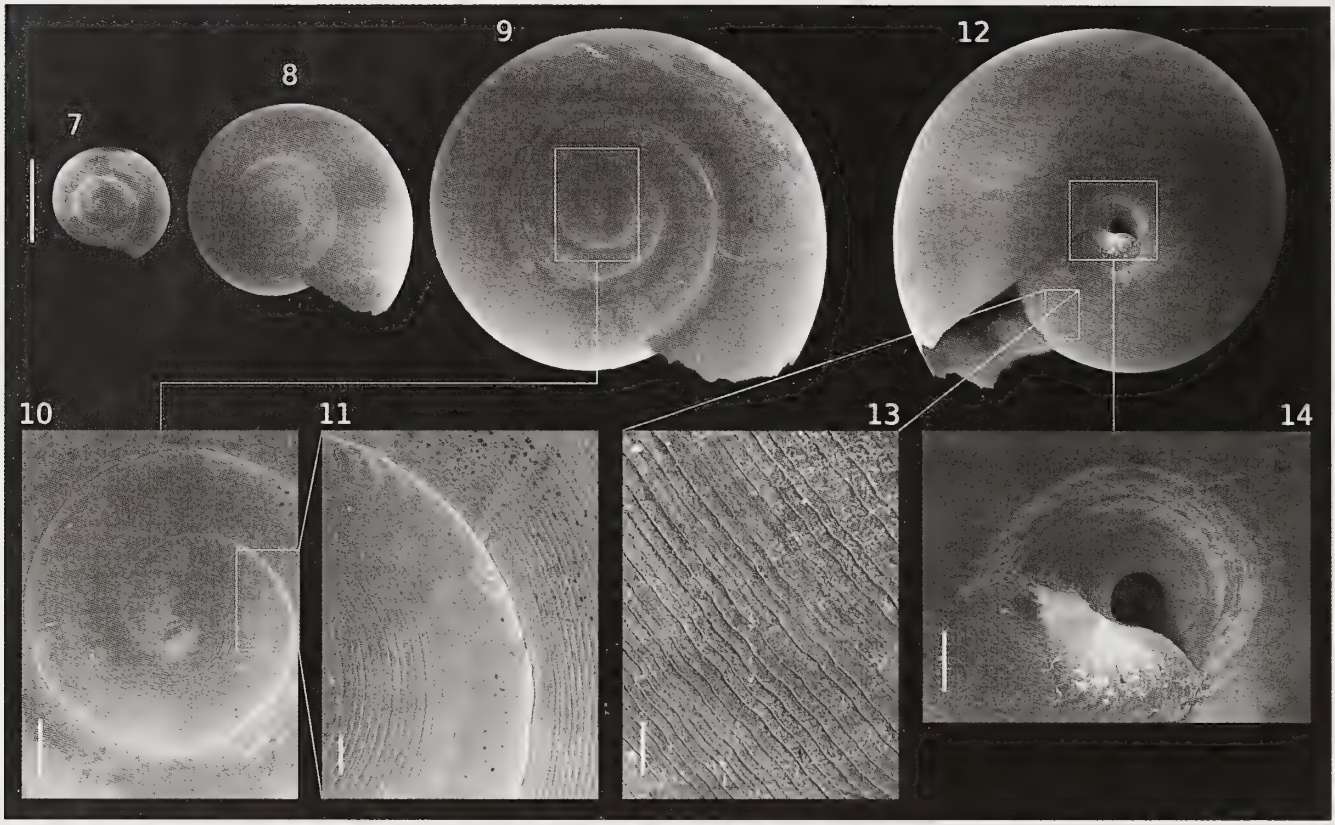
the ratios of: shell height to shell width (H/W), height of aperture to width of aperture (Ha/Wa), shell width to width of aperture (W/Wa), shell height to height of spire (H/Hs), and shell height to height of last whorl (H/Hl). Original data were standardized and size-adjusted using the Burnaby (1966) method. A principal component analysis was conducted. The curve of expected eigenvalues of a random model (Broken Stick) was used to select significant components. Eigenvalues for components under this curve were considered non-significant and then discarded (Jackson, 1993). All statistical procedures were performed with PAST software (Hammer et al. 2001).

Detailed external morphology and gross anatomy were examined in laboratory using a stereo-microscope Zeiss Stemi SV 11 with attached *camera lucida*. Dissections were performed using standard techniques, with specimens immersed in fixative under the stereo-microscope. Organs of the pallial cavity, reproductive, digestive, circulatory, excretory, and nervous systems were investigated. Radulae and jaw were manually extracted and prepared by immersion in a 10% solution of sodium hypochlorite for 24h, and later rinsed in distilled water. Nineteen specimens were dissected, from which six had the radulae and jaw removed for imaging.

Images were taken using a Canon SX170 IS digital camera and SEM photography using a JEOL JSM-6510 at "Laboratório de Imagens em Microscopia Óptica e de Varredura (LABIM-UFRJ)", at Department of Zoology, UFRJ.

Anatomical comparisons of the reproductive system were performed against taxa illustrated in Schileyko (2002a, b), Sutcharit and Panha (2008), and Hyman and Ponder (2010). Only species with similar anatomy and detailed information available were included on Discussion.

Anatomical abbreviations used are: **A,** anterior; **ag,** albumen gland; **aga,** parietal ganglia; **ao,** aorta; **au,** auricle; **bc,** bursa copulatrix; **bga,** buccopharyngeal ganglia; **bm,** buccal mass; **br,** retractor muscle of buccal mass; **ca,** caudal apparatus; **car,** carrefour; **cg,** capsule gland; **cga,** cerebral ganglia; **dg,** digestive gland; **ep,** epiphallus; **ga,** genital atrium; **gv,** gastric vein; **her,** hermaphroditic duct; **il,** intestinal loop; **ja,** jaw; **ki,** kidney; **lga,** pleural ganglia; **me,** mantle edge; **mga,** mandibular ganglia; **ml,** mantle lobes; **ms,** mouth slit; **nc1–nc5,** nerves of the cerebral ganglia; **np1–np6,** nerves of the pedal ganglia; **oc,** ocular tentacle; **oe,** oesophagus; **or,** oral tentacle; **ov,** oviduct; **ovt,** ovotestis; **pc,** pericardium; **pe,** penis; **pec,** epiphallic



Figures 7–14. *Ovachlamys fulgens*. Shells. **12–14.** IBUFRJ 21217. **7–9.** Growth series, apical view. **7.** IBUFRJ 21218. **8.** IBUFRJ 21219. **9–11.** IBUFRJ 21221. **10–11.** Details of protoconch and external microsculpture. **12.** Adapical view. **13–14.** Details of external microsculpture and umbilical perforation. Scale bar for 7–9, 12=1 mm; 10, 14=200 μm; 11, 13=50 μm.

caecum; **pga,** pedal ganglia; **pn,** pneumostome; **po,** protractor muscle of the buccal mass; **pr,** prostate; **prm,** penial retractor muscle; **ps,** penial sheath; **pu,** primary ureter; **pv,** pulmonary vein; **re,** rectum; **ro,** retractor muscle of the odontophore; **rs,** radular sac; **sd,** ducts of salivary gland; **sg,** salivary gland; **st,** statocyst; **sto,** stomach; **su,** secondary ureter; **ta,** talon; **ut,** uterus; **ut',** second region of the uterus; **vd,** vas deferens; **ve,** ventricle; **vg,** vagina; **vga,** visceral ganglia; **vs,** vascular system.

RESULTS

Systematics

Helicarionoidea Bourguignat, 1877
Helicarionidae Bourguignat, 1877

Ovachlamys Habe

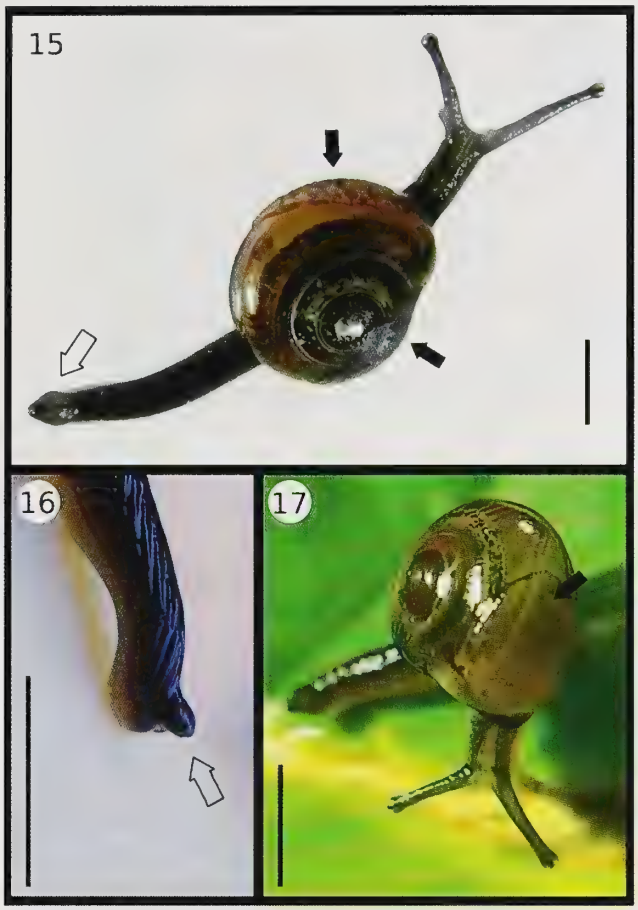
***Ovachlamys fulgens* (Gude, 1900)**
(Figures 2–30)

Macrochlamys fulgens Gude, 1900: 75, pl. 8, figs 24–26.

Description: Shell (Figures 3–4) globose, dextral, very fragile, translucent, reddish to pale golden. Shell surface micro-sculptured with undulating spiral grooves. Whorls 2–4, regularly increasing in diameter. Last whorl rounded or somewhat shouldered. Umbilicus deep, partially filled by a small reflection of the peristome. Aperture crescent-shaped, with simple lip.

External Morphology: Soft parts (Figures 15–17): External surface grades from light-brown to dark-gray during ontogeny. Pedal sole tripartite. Cephalopedal mass mainly built from a series of oblique muscles. Two pairs of cephalic tentacles. Mantle edge extended giving rise to two flattened lobes, which cover part of the shell when the animal is active. Caudal apparatus consisting of curled-up posterior part of pedal sole, caudal horn large, nearly heart-shaped. Caudal groove present.

Pallial Cavity, Circulatory and Excretory Systems (Figures 18–20): Pallial cavity small, short, nearly rectangular in shape. A secondary ureter crosses pallial cavity and opens to exterior close to pneumostome. Rectum large, running close to but not attached to secondary ureter, opening outside pallial cavity. Vascular system with large and well defined veins. Pericardium small, discernible on fresh specimens. Auricle fibrous, translucent, connected to a pulmonary vein that enters pallial cavity. Ventricle highly muscular, opaque, small, half size of auricle, connected to short aorta. Aorta subdivided into cephalic and gastric veins, former hardly visible, latter evident, with vascular ramifications on digestive gland.



Figures 15–17. *Ovachlamys fulgens*. Living specimens. **15.** General external morphology. **16.** Detail of caudal apparatus. **17.** Detail of mantle lobe lining the shell; pulmonary vein and rectum are visible through the shell. Empty and filled arrows indicate the caudal horn and mantle lobe, respectively. Scale bar=2 mm.

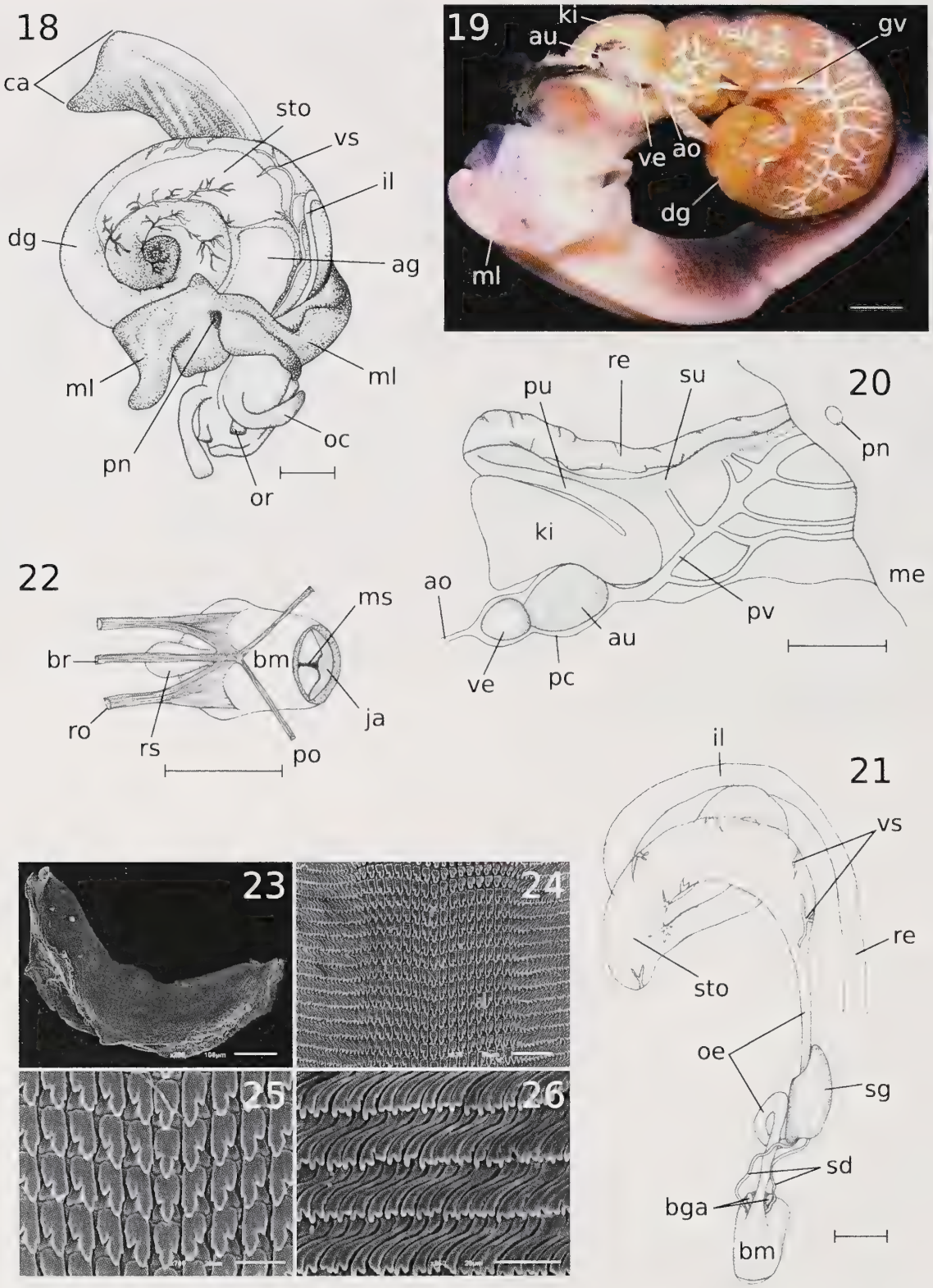
Kidney rectangular to trapezoidal, corresponding to about 40% of pallial cavity size. Primary ureter large, narrowing toward secondary ureter. Nephridiopore opening to exterior close to pneumostome. Kidney and ureters internally folded.

Digestive System (Figures 21–26): Mouth a vertical slit in antero-ventral margin of snout. Buccal mass rectangular. Odontophore about half of buccal mass volume, subdivided into two elliptical bulbs. Two pairs of muscles insert at each odontophore bulb: one strong and large retractor muscle inserted at posterior region runs backward, and one thinner protractor muscle inserted at posterior region of bulb runs obliquely toward lateral body wall. Thin retractor muscle, inserted ventrally, near center of buccal mass, runs backward between odontophore bulbs. Radular sac projected posteriorly, with about half size of buccal mass. Oesophagus long, medium-dorsally inserted at buccal mass. Crop absent. One pair of salivary glands fused, overlaying oesophagus latero-dorsally. One pair of narrow and thin walled ducts connects salivary glands to buccal mass near buccopharyngeal ganglia. Stomach large and elongated, with thin walls, dorsal to visceral mass. Stomach connects to ventral, S-shaped, intestine. Both intestine and stomach partially embedded in granular digestive gland, which occupies the remaining volume of the spire. Anus opening close to pneumostome.

Radula and Jaw: Radulae with 127 teeth per row (55-(8)-1-(8)-55), each row measuring about 25 μm. Central tooth symmetric tricuspid, rectangular, mesocone large, lanceolate, ectocones pointed, located on about 2/3 of tooth length. Lateral teeth asymmetric, tricuspid; mesocone large, lanceolate; endocone small, blunt, placed near tooth tip; ectocone, triangular, pointed, located on middle of tooth. Marginal teeth sinoidal and elongated, bicuspid, endocone slightly longer than ectocone. Jaw smooth and crescentic, without central cusp.

Reproductive System (Figure 27): Ovotestis fragile, with at least three distinct lobes; hermaphroditic duct almost straight, convoluted towards small talon, which is embedded in albumen gland. Carrefour rounded to ovate, close to talon, partially immersed in albumen gland. Albumen gland bean-shaped, with corrugated wall touching stomach. Prostate and uterus running alongside. Uterus spacious, with large lobules, and subdivided in two regions, one with prostate partially embedded and other expanded below albumen gland, grading in size during ontogeny. Capsule gland hardly visible, located close to carrefour between uterus and albumen gland, totally immersed in latter. *Bursa copulatrix* small and sacculiform, inserted on vagina by short duct. Penial sheath present. Penis long, cylindrical and slightly compressed in transversal section. Epiphallus small and narrow, about 1/5 of penial length. Penial retractor muscle attached to tip of epiphallic caecum. Vas deferens emerging from prostate gland, connected at junction of penis and epiphallus. Penis and vagina inserting side-by-side in long genital atrium.

Nervous System (Figures 28–30): Pairs of cerebral, pleural, parietal, and pedal ganglia connected and partially fused, forming asymmetrical nervous ring. Visceral ganglion on left side only. Five nerves running from each cerebral ganglion (nc1-nc5), two from ventro-lateral region (nc1–nc2), one connects to tip (nc1) and the other to the base (nc2) of ocular tentacles. The remaining three cerebral ganglion nerves (nc3–nc5) run from anterior region and connect, respectively, to oral tentacle, to mandibular ganglion, and to buccopharyngeal ganglion. Pair of pedal ganglia gives rise to several delicate nerves running ventrally towards pedal sole (np1–np6), but exact end target of those nerves in pedal sole not determined. One statocyst immersed in each pedal ganglion. Two additional pairs of ganglia are connected to nervous ring: one pair of buccopharyngeal ganglia close to pharynx, on dorso-lateral surface of buccal mass; and one pair of mandibular ganglia near distal end of buccal mass.



Figures 18–26. *Ovachlamys fulgens*. **18–19.** External morphology. **18.** Apical view, shell removed. **19.** Lateral view, digestive gland slightly turned up to expose the gastric venation and kidney partially removed to expose the heart. **20.** Pallial cavity and associated organs. **21.** Digestive system. **22.** Buccal mass, ventral view. **23.** Jaw. **24.** Radular teeth. **25.** Close-up to show the central, lateral and (**26**) marginal teeth. Scale bar for 18–22 =1 mm; 23=100 μm; 24=50μm; 25–26=20 μm.

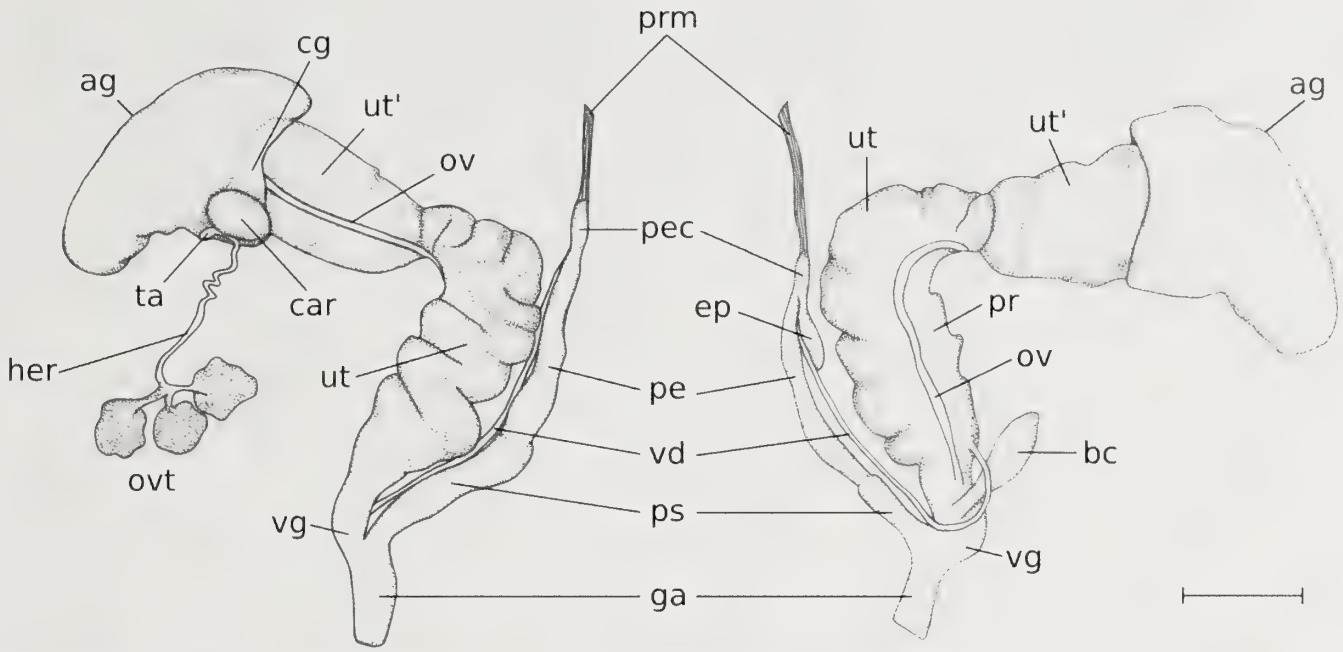


Figure 27. *Ovachlamys fulgens*. Reproductive system, view from two sides. Scale bar=1 mm.

Morphometric Analysis (Figure 2, Tables 1–3): Only the first two components were considered significant. No segregation in clusters were observed in the scatter plot of voucher specimens collected in PARNASO and syntypes of *O. fulgens* ordinated along the PC1 and PC2. The Component 1 was most influenced by the ratios of the shell height to height of spire (H/Hs) and the shell height to height of last whorl (H/Hl). The Component 2 was most influenced by the ratio of the shell width to width of aperture (W/Wa).

Type Material: Field Museum of Natural History, FMNH 43045, syntype, 1 dry shell; Natural History Museum, London, U.K., NHMUK 1922.8.29.12, syntype, 1 dry shell.

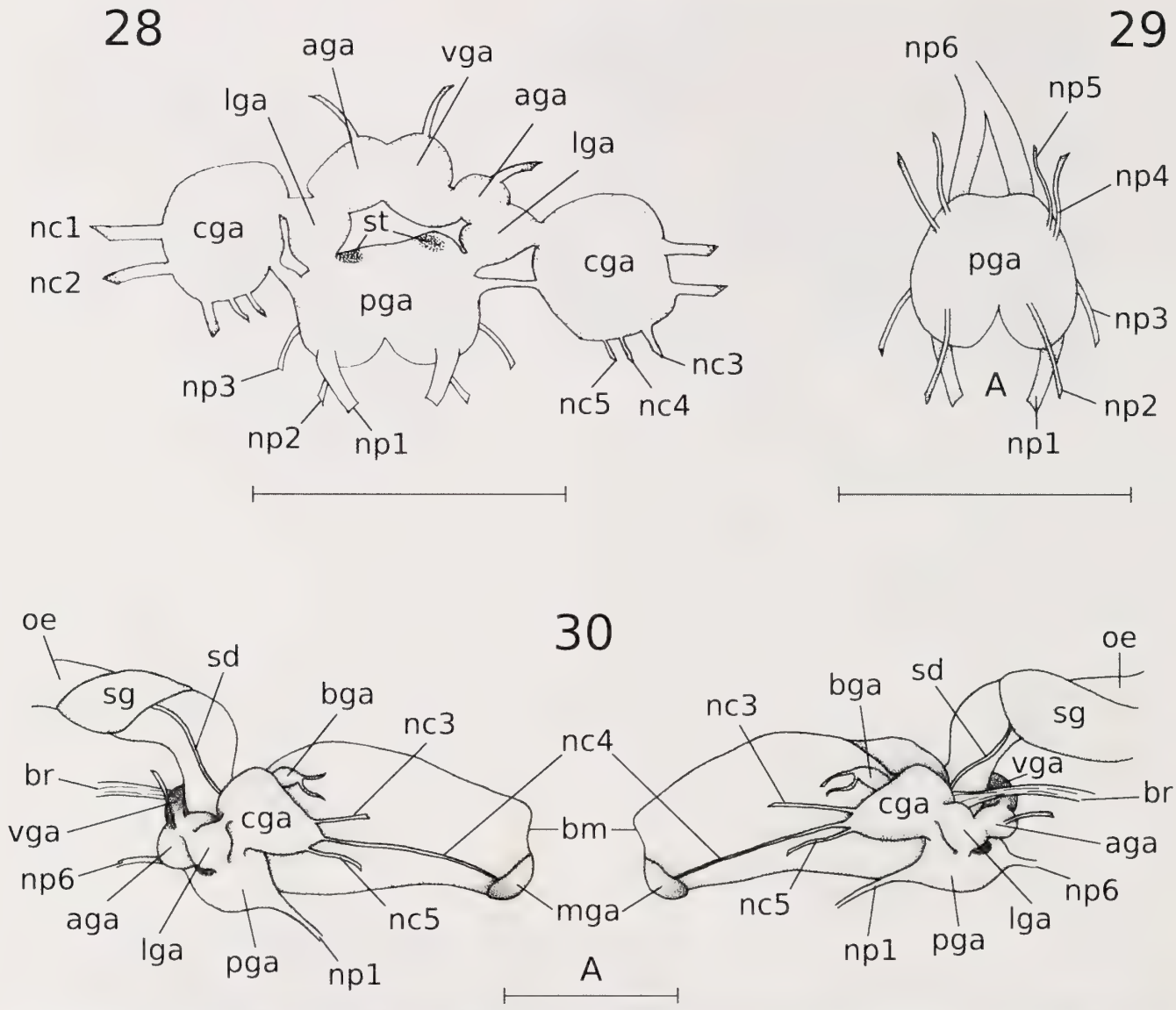
Type Locality: Loo Choo Islands, Japan.

Other Material Examined: Brazil, Rio de Janeiro, PARNASO: Guapimirim: IBUFRJ 21217 (1 shell), IBUFRJ 21218 (1 shell), IBUFRJ 21219 (1 shell), IBUFRJ 21220 (1 shell), IBUFRJ 21221 (1 shell), IBUFRJ 21222 (1 shell), IBUFRJ 21225* (93 specimens), IBUFRJ 21229 (4 specimens). Teresópolis: IBUFRJ 21226 (1 specimen), IBUFRJ 21236 (20 shells). Lot with dissected specimens marked by an asterisk.

Behavior: Activity of *O. fulgens* in PARNASO seems to be correlated with high humidity and all live specimens were collected at night or after rainfall, always near the trails edge, inside the park. Most specimens were found on herbaceous vegetation (e.g. *Hibiscus* sp., *Quesnelia* sp., and *Heliconia* sp.) about 100–150 cm above ground. Some live specimens were also found in leaf litter, on exposed rocks, on green moss, and on fallen trunks.

When disturbed, the specimens started the typical behavior under stress conditions exhibited by this species, for which it is popularly known as the jumping snail. In such behavior, the animal retracts the head inside the shell and turns the pedal sole, including the caudal horn, in an abrupt and repeatedly circular movement (usually accompanied by an increase in mucous secretion). Most likely this activity is intended to discourage predators and often results in the snail falling from leaves to the ground.

Such behavior represents a peak of high metabolic rates and comes at a cost. After a short period of intense activity with usually two or three cycles of intermittent jumps (each cycle takes about 2 seconds long) the animal often becomes sluggish and appears exhausted. The presence of a powerful vascular system with large vessels certainly assists in promptly supplying the body needs of energy and oxygen during the jumps, but the small sized pallial cavity seems counterintuitive in providing the necessary amount of oxygen. We suppose the presence of body extensions may assist this need. Such extensions are most often observed in marine gastropods (e.g. Cypraeidae and Ovulidae) as projections of the foot and are associated with camouflage, shell repair, anti-predatory activities, etc. (Vermeij, 1978). In *O. fulgens* the mantle edge extends forming two thin, wide lobes, each one lies externally on each side of the shell. These lobes presumably assume a gas exchange function. Although further studies are required, structures with increased surface area to volume ratio are usually associated to exchange with



Figures 28–30. *Ovachlamys fulgens*. Nervous system. **28.** Nervous ring, cerebral commissure cut; **29.** Pair of pedal ganglia, ventral view. **30.** Lateral view, right and left side, respectively; nerves from the cerebral ganglia (nc1 and nc2) and nerves nerves from the pedal ganglia (np2–np5) not illustrated. Scale bar=1 mm.

environment, mainly to gas exchange in terrestrial organisms (Schmidt-Rhaesa, 2007). Since the presence of such lobes increases the water loss by the animal, the foraging activity is limited to high humidity periods and spots.

DISCUSSION

Shell Morphometrics: Anatomical data were absent in the original description of *Ovachlamys fulgens*. Based solely on shell attributes, no significant differences were found among between the syntypes (photos) of *O. fulgens* and the voucher specimens from PARNASO. It reinforces the conclusion that the record of this species to PARNASO is not based on a misidentification. The most discriminative features among the quantitative descriptors used (Table 3) are the globose shell and the wide aperture. Those features emerge as important conchological characteristics for the taxonomy of *O. fulgens*.

Morphology: Reproductive System: One remarkable feature of *O. fulgens* is the uterus subdivided in two distinct regions. The region close to the albumen gland is reduced or even inconspicuous in young specimens, becoming as large as the albumen gland in adults.

Among Ariophantidae, *Macrochlamys petrosa* and *Sarika asamurai* differ from *O. fulgens* by the very convoluted hermaphroditic duct, the short genital atria, and the presence of flagellum and stimulator. Additionally, in *M. petrosa* the *bursa copulatrix* is large and

Table 1. *Ovachlamys fulgens*. Morphometrics. Unprocessed values: minima, maxima, means, and standard deviations. Values in millimeters. Total of 20 analyzed specimens from PARNASO and two syntypes. Measured variables: shell height (H), shell width (W), height of spire (Hs), height of last whorl (Hl), height of aperture (Ha), width of aperture (Wa), shell height to shell width (H/W), height of aperture to width of aperture (Ha/Wa), shell width to width of aperture (W/Wa), shell height to height of spire (H/Hs), and shell height to height of last whorl (H/Hl).

	Minimum	Maximum	Means	S.D.	Syntype FMNH	Syntype NHMUK
H	2.73	4.86	3.80	0.52	4.85	4.86
W	3.99	6.81	5.47	0.68	6.57	6.81
Hs	0.36	0.75	0.50	0.10	0.59	0.70
Hl	2.36	4.26	3.30	0.46	4.26	4.17
Ha	2.16	3.28	2.79	0.28	3.28	3.25
Wa	2.13	3.56	2.93	0.35	3.44	3.56
H/W	0.65	0.74	0.69	0.02	0.74	0.71
Ha/Wa	0.87	1.05	0.95	0.05	0.95	0.91
W/Wa	1.82	1.92	1.87	0.03	1.91	1.92
H/Hs	5.39	10.51	7.69	1.18	8.23	6.92
H/Hl	1.11	1.22	1.15	0.03	1.14	1.17

elongated, with a long duct, and the epiphallus is of almost equal size to that of the penis. In *S. asamurai*, the *bursa copulatrix* is sacculiform and elongated, with a short duct, and the epiphallus is about three to four times longer than the penis. Among the Helicarionidae, *Helicarion cuvieri* and *Nitor circumcincta* differ from *O. fulgens* by the presence of a flagellum. Additionally, in *H. cuvieri* the *bursa copulatrix* is spherical and large, with a duct as long as the bursa, the epiphallic caecum is absent, and the epiphallus is slender and about two times longer than the penis. In *N. circumcincta*, the talon is free (not immersed in albumen gland), the hermaphroditic duct is very convoluted, the *bursa copulatrix* is elongated and indistinct from the duct, the penis is enlarged close to the penial sheath, and the epiphallus is of same size as the penis, narrowing towards the flagellum.

Nervous System: The mandibular and the buccopharyngeal pairs of ganglia were not previously reported for the family. Both are extra to the nervous ring and directly connected to the cerebral ganglia.

The asymmetrical structure of the nervous ring is determined by the visceral ganglion present on the left side only. The main differences in nervous ring between *O. fulgens* and the close-related species *Macrochlamys petrosa* (Hutton, 1834) (illustrated in Hyman and Ponder, 2010: 32) are the larger thickness of the commissures among the cerebral, pleural and pedal ganglia in *M. petrosa*; and the pair of pedal ganglia, which is partially fused in *O. fulgens* and separated in *M. petrosa*.

Hausdorf (1998) noticed the absence of direct innervation from cerebral ganglion to the penis as a nonconvergent feature and a possible synapomorphy for Ariophantidae. To recognize the boundaries among the families of Helicarionoidea is beyond the scope of the present study. But it is noteworthy that the cerebral innervation of the penis was not found in any specimens of *O. fulgens* studied here, which triggers the need for further studies and questions the validity of the placement of *O. fulgens* among Helicarionoidea.

Table 2. *Ovachlamys fulgens*. Summary table of PCA analysis.

PCs	Eigenvalue	% variance
1	3.09004	58.177
2	1.2235	23.035
3	0.656144	12.353
4	0.297415	5.5995
5	0.031913	0.60083
6	0.0102383	0.19276
7	0.0016076	0.030266
8	0.0005785	0.010892
9	3.6403E-05	6.8537E-04
10	1.09737E-05	2.066E-04
11	7.03218E-16	1.324E-14

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Table 3. *Ovachlamys fulgens*. Component loadings for each variable.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11
H	0.091972	0.025714	0.070687	-0.046637	0.014812	-0.12938	0.46067	0.113	0.14198	-0.74252	0.41264
W	0.01331	-0.10904	-0.11811	0.032733	-0.021614	0.0019449	-0.11013	0.44066	-0.76835	0.06573	0.41406
Hs	-0.33936	0.18106	0.15364	0.15458	0.047074	0.80676	0.11285	-0.18812	0.0034532	0.0482	0.31784
Hl	0.17965	-0.0077193	0.054471	-0.081263	-0.025868	-0.22881	0.56131	-0.17176	0.081207	0.62971	0.39669
Ha	0.19632	-0.087774	-0.058934	0.48253	-0.031684	-0.20487	-0.38764	-0.60639	-0.05419	-0.11444	0.37479
Wa	0.0021474	-0.20558	-0.034111	0.071171	-0.04283	0.037495	-0.38112	0.49116	0.60843	0.15926	0.4077
H/W	0.31489	0.48139	0.70018	-0.25606	0.057443	-0.056812	-0.29927	0.037357	-0.058	0.019415	0.10982
Ha/Wa	0.35664	0.34911	-0.012772	0.72857	-0.043937	0.070518	0.21659	0.33019	0.030757	0.059089	-0.2295
W/Wa	0.088697	0.65692	-0.66255	-0.26172	0.073018	0.0066025	-0.12573	-0.046389	0.072927	0.0031889	0.15637
H/Hs	0.52713	-0.2899	-0.10117	-0.10714	0.72597	0.29406	0.0067254	-0.029504	-0.0004816	-0.0089564	-0.043445
H/Hl	-0.54157	0.18826	0.10113	0.22801	0.67528	-0.37676	0.0035608	0.075902	0.010777	0.055362	0.046207

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Research Note

First occurrence of a novel *Corbicula* (Bivalvia: Corbiculidae) Form D lineage in the Ohio River, USA

Additional Keywords: Asian Clam, invasive species, invasion, exotic species, non-native species

Asian Clams (Genus *Corbicula*) are moderately-sized (typically <50 mm) freshwater bivalves native to the temperate/tropical regions of Africa, southern Asia, and eastern Australia (Britton and Morton, 1986). *Corbicula* was first recorded in North America in 1924 in British Columbia, Canada, and breached the Continental Divide in the late 1950s (Bates, 1962; Fechtner, 1962; Stein, 1962; Counts, 1986). Since then, it has spread throughout the United States and into South America (Lee et al., 2005).

Literature reports vary from an invasion of only a single *Corbicula* species to invasions of multiple taxa (Counts, 1986; Hoagland, 1986; Lee et al., 2005). However, *Corbicula* taxonomy is muddled and unclear, as is the number of species that have become established in North America (Hillis and Patton, 1982; Hoagland, 1986). *Corbicula* species appear to switch reproductive strategies and become androgenic clones when they invade new ecosystems outside of their native range (see Lee et al. [2005] and references therein; Hedtke et al., 2011; Pigneur et al., 2011; 2014). This complex reproductive behavior can include not only hermaphroditism but also capturing eggs of other *Corbicula* lineages and potentially replacing maternal nuclear DNA. Separate *Corbicula* lineages can differ in their nuclear 28S ribosomal genotype yet share identical mitochondrial mtDNA genotypes (Lee et al., 2005; Tiemann et al., 2017). This phenomenon contorts our understanding of traditional species concepts and makes it difficult to determine if *Corbicula* taxa are either separate species or individuals of distinct phenotypes belonging to a single polymorphic species (Britton and Morton, 1986; Lee et al., 2005).

Hillis and Patton (1982), based on electrophoretical and morphological data, suggested two *Corbicula* "forms" occur in North America. Shortly thereafter, Britton and Morton (1986) proposed the Form morphotype classification and recognized two *Corbicula* forms in North America—Form A and Form B. Later, Lee et al. (2005) expanded on the Form concept and reported three forms in the New World—Forms A and B in North America and

Figure 1. Location of where Tiemann et al. (2017) reported *Corbicula* Form D (star) and where it was collected in the Ohio River, near Newburgh, Indiana (hexagon).

Form C in South America. Morphometrically, small (<20 mm) Forms A and B specimens can be distinguished via nacre color—Form B has a deep purple nacre whereas Form A has a white nacre with purple highlights (Hillis and Patton, 1982; Britton and Morton, 1986). However, these morphological differences become unreliable on larger specimens (Britton and Morton, 1986).

The Midwest had long been recognized as having only one *Corbicula* taxon, *Corbicula fluminea* (=Form A), until recently (Cummings and Mayer, 1992). Tiemann et al. (2017) reported three distinct *Corbicula* taxa occurring in Illinois, including one that had never been documented in the New World. The newest taxon, referred to as *Corbicula* Form D, has fine rust colored rays on the periostracum, white nacre with purple teeth, and external ridges that are not as pronounced as other *Corbicula* taxa. *Corbicula* Form D was known from only a small stretch of the Illinois River in northern Illinois, but data were lacking as to other locations. Herein, we report on an additional location of *Corbicula* Form D.

On 24 October 2017, one live individual of *Corbicula* Form D (Figure 1) was collected in the Ohio River in the Newburgh Pool upstream of Rockport, Indiana at river mile 744.4 (Latitude 37.9111° N, Longitude 87.0123° W). This specimen (Figure 2) was deposited for accession in the Illinois Natural History Survey Mollusk Collection, Champaign (catalogue number INHS 87523). It should be noted that the primary objective of this field outing to the Ohio River was to conduct unionid sampling and *Corbicula* were not the primary focus. Therefore, we cannot assess density or relative abundance of the *Corbicula* here, and are only reporting presence via incidental encounter.

Accurate species identification and reporting is critical in monitoring the spread and, ultimately, minimizing the ecological and economic effects of this novel *Corbicula* form (Tiemann et al., 2017). We are hesitant to speculate as to when and how Form D arrived in the Ohio River. Because of logistical constraints (e.g., time, effort, and monetary costs), *Corbicula* from navigable rivers, like the Ohio River, are likely to be underrepresented during field surveys and in museum collections. If not overlooked, *Corbicula* are often simply noted as "present" with no discernable differentiation among taxa when conducting

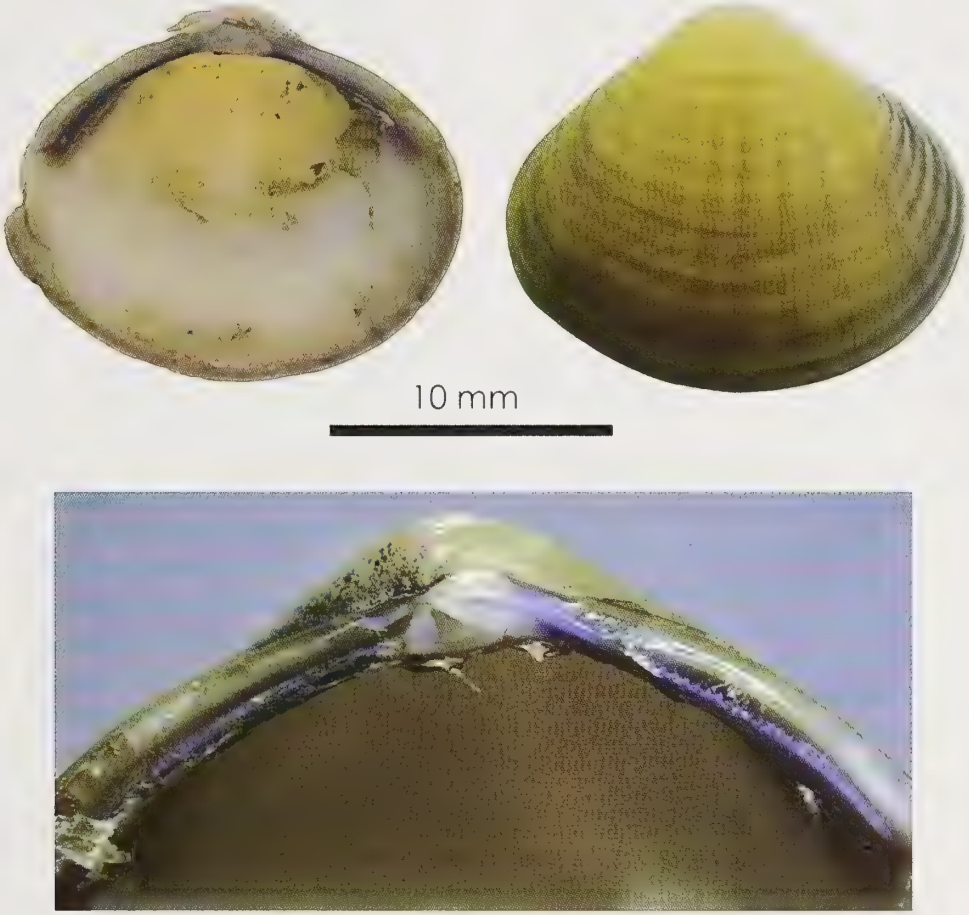

Figure 2. *Corbicula* Form D, internal view of right valve (top left) and external view left valve (top right) and hinge of left valve (bottom). Collected from the Ohio River, near Newburgh, Indiana (INHS 87523). This specimen is 18 mm in length. Distinguishing characteristics for *Corbicula* Form D include fine rust colored rays on the periostracum and external ridges that are not as pronounced as other *Corbicula* taxa.

field surveys. Natural history museums (e.g., INHS Mollusk Collection) do have *Corbicula* specimens, but these are often larger specimens, which can be unreliable for morphotype discrimination. Regardless, *Corbicula* Form D is known from a larger area than reported by Tiemann et al. (2017). It seems likely that it has gone unnoticed in other areas, including the Mississippi River, which is a likely dispersal pathway between the specimen reported by Tiemann et al. (2017) and the Ohio River specimen we are reporting. With the aid of external funding, additional field surveys will help determine the validity of this claim.

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Research Note

Flabellina McMurtrie, 1831: The earliest valid Latinization?

Authorship and dating of the nudibranch genus-level name *Flabellina* is dependent upon the first Latinization of the French vernacular name 'Les Flabellines' introduced and used by Cuvier (1830) in the second edition of his *Le règne animal*... Lemche (1964) traced a Latinization to Voigt (1834: 124) in the German edition of Cuvier's work and, in due course, *Flabellina* Voigt, 1834 was added to the Official List of Generic Names in Zoology, as Name 1721 (ICZN, 1966).

However, following Winckworth (1941) and Cowan (1969a; 1969b), Burn (2006) attributed *Flabellina* to Griffith and Pidgeon, 1833, certainly to December of that year, but possibly as early as September (Cowan, 1971). The online World Register of Marine Species (Caballer et al., 2014) and MolluscaBase (2018) as well as the recent taxonomic re-assessment of the family Flabellinidae (Korshunova et al., 2017) list J.E. Gray, 1833 as the author of *Flabellina*, an incorrect attribution, as Gray had no responsibility for the English edition of Cuvier's text (Petit and Coan, 2008; Petit, 2012). J.C. Smith (1993: 188) noted a translation into English by McMurtrie (1831) as "the basis of most of the English translations" of Cuvier. Reference to McMurtrie (1831) reveals that the latinized genus name *Flabellina* occurs on page 343 of volume 2 (Figure 1), the title page of which is dated 1831. This year is confirmed in the publication registration statement printed on the reverse of the title page: "Entered according to the act of congress, in the year one thousand eight hundred and // thirty one, by G. + C. + H. Carvill, in the clerk's office of the southern district of // New York."

Despite his Latinization of the vernacular name, McMurtrie still considered Cuvier as the author of *Flabellina*. But in fact that name should be credited to McMurtrie alone as he was the first to adopt it. "Adopt", as defined in the glossary of the International Code of Zoological Nomenclature, means "To use an unavailable name as a valid name of a taxon in a way which established it as a new name with its own authorship and date" (ICZN, 1999: 99).

The short timeframe between publication of Cuvier (1830) and McMurtrie's English edition (1831) makes it highly unlikely that an earlier valid Latinization will be found (but see note in Acknowledgments below). *Flabellina* McMurtrie, 1831 thus appears to encompass the earliest Latinization, author, and date. *Doris affinis* Gmelin, 1791, the only species included by Cuvier (1830) in his "Les Flabellines", was faithfully translated as the only species in *Flabellina* by McMurtrie (1831). *Doris affinis* Gmelin, 1791 is therefore type species, by monotypy, of *Flabellina* McMurtrie, 1831.

Accordingly, corrections now need to be made to the entry on the Official List of Generic Names in Zoology, and on the MolluscaBase/World Register of Marine Species website.

GASTEROPODA NUDIBRANCHIATA. 343

FLABELLINA, Cuv.

The tentacula of the Eolidæ, with radiating rectiform branchiæ, supported by five or six pedicles on each side; they are closely allied to the Glauci, and in fact to all the Nudibranchiata, whose branchiæ are situated on the sides of the back(1).

(1) *Doris affinis*, Gm., Cavol., Polyp. Mar., VII, 4.

Figure 1. McMurtrie's (1831) Latinization, genus description (at top of page 343) and type designation of *Flabellina* (at bottom of page 343). The intervening text, containing translation of other genera and their footnotes, has been excluded.

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Correspondence with Charles Cowan and Richard Petit, both regrettably deceased, concerning the Griffith and Pidgeon translation of Cuvier (1830) was informative and greatly appreciated. Gemma Steele, Librarian, Museums Victoria, and other library staff, exceeded all expectations by securing the McMurtrie translation, and those references beyond my personal library. Gary Rosenberg (in litt.) provided a reference to an earlier latinization of 'Les Flabellines' (Van der Hoeven 1830), but this was unaccompanied by a description or bibliographic reference, and is therefore a *nomen nudum* not altering the information presented above. He also noted that no other names of mollusks appear to have been introduced in McMurtrie's work. I am also grateful for the helpful comments of an anonymous reviewer. Richard Willan graciously prepared and presented this note for publication.

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